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FACTORS RELATING TO THE CONTROL OF ORDINARY TOBACCO MOSAIC[1]

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INTRODUCTION

Certain factors involved in the inactivation and survival of the ordinary tobacco mosaic virus (*tobacco virus 1*) when in close association with the soil have been discussed in a recent paper from this laboratory (*5*).[2] The present paper deals more particularly with the survival of the virus in dead host tissues outside of the soil, and with the relation of virus harbored both in the soil and outside of the soil to infection of and dissemination in succeeding crops.

The problem of tobacco mosaic control is complicated by a number of circumstances not easily eliminated as possible factors in the development of the disease. Despite the apparent abundance of potential sources of infection and means of dissemination, satisfactory explanations for many instances and variations of disease occurrence have been difficult to obtain. Most of the data relating to the subject are based upon field observations and experiments, which, though highly desirable, may by themselves lead to a misinterpretation of results. In the present investigations an attempt has been made to arrive at conclusions chiefly through more exacting laboratory methods, although these studies have been supplemented by 10 years of field records.

The results of this investigation along with those reported in the earlier paper suggest certain bases which need to be taken into consideration in the practical control of the disease, as well as in future field surveys and experiments which may be undertaken for the purpose of determining the relative importance of the various factors concerned.

THE PRESENT STATUS OF THE PROBLEM

The common yet sporadic occurrence of mosaic in the field, together with the doubtful nature of the causal agent, has resulted in some skepticism and uncertainty about control methods which are difficult to overcome. The literature offers a wide variety of ideas on cause and control which need not be cited in the present discussion. Some of the earliest observers, however, noted the apparent relation of infested soil to the disease, as pointed out in a prior paper from this laboratory (*9*), in which these early observations were further corroborated. Though it was obvious that the soil itself was not the

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[2] Reference is made by number (italic) to Literature Cited, p. 272.

only source of infection for mosaic, the possibilities of other sources of infection and means of dissemination have been emphasized to such an extent that considerable confusion may be said to exist. Thus, the importance attached to perennial host plants and insect transmission in relation to this virus has receded only slowly, whereas the belief that the virus is commonly spread through the use of infested tobacco by workmen in the growing crop, as emphasized by Valleau and Johnson (*13*), has gained considerable support.

The present status may be well represented by Wolf's (*14*) recent discussion of tobacco mosaic in the flue-cured tobacco district of the United States. In a 3-year survey it was found that the mosaic in 125 fields out of 229 examined could be traced to infested soil alone. Sixty-three of the fields were infected from both diseased seedlings and infested soil; 24 from infected seedlings alone; and in 17 the source could not be definitely determined. These data seem to establish the preponderance of infection from the soil. However, in a later paragraph Wolf states on the basis of the survey that "the careless use of manufactured tobacco constitutes the most common source of inoculum for mosaic in the flue-cured areas." Lehman (*10*), reporting on field experiments in the flue-cured district, apparently attaches somewhat less importance to direct infection from either the soil or the use of infested tobacco by workers, but finds high percentages of mosaic spread resulting from cultivation.

Without going further into the present status of the problem, it is evident that there is a need for more detailed study of the fundamental questions involved, together with subsequent thorough field surveys, taking all circumstances into consideration, before certain and efficient control methods may be devised for the different methods of tobacco culture practiced.

METHOD OF INVESTIGATION

The value of the present studies depends largely upon utilizing the "local-lesion" method of roughly determining the amount of virus present in a given sample of tissue (fig. 1). Degree of accuracy in sampling, inoculation, and counts has often been sacrificed for time and space in order to cover the field in a preliminary manner. However, approximate determinations by the local-lesion method, even though freely estimated, are far more significant under the circumstances than methods of determination previously employed. Accurate technique, together with the statistical method of approach, can and should no doubt be employed in further experiments dealing with the more significant results in need of verification.

The laboratory or greenhouse work was conducted in essentially the same manner as previously described (*5*) with respect to sampling of the plant parts or the soil for the virus. The variable nature and condition of plant parts made it impractical at times to attempt to secure accurate comparisons, and consequently only estimated comparative volumes of the respective tissues were taken for extraction with 5 cc of water for leaf tissue or 10 cc for roots, stalks, etc., except as otherwise mentioned. The dry weight of root and stalk samples averaged about 2 and 5 g, respectively. Plant parts were crushed in water with mortar and pestle and allowed to extract for 1 to 2 hours. The soil samples made with a cork borer were more strictly comparable in volume. Soil samples were merely shaken up twice in a test tube,

the coarser soil allowed to settle out, and the extract decanted off. These extracts were then rubbed over five or six leaves (5 to 10 inches long) of the hybrid *Nicotiana glutinosa* L. × *N. tabacum* L. (7) with

FIGURE 1.—Local lesions on the hybrid host (*Nicotiana tabacum* × *N. glutinosa*, F_1 generation) showing typical spotting resulting from wiping infectious extract over the leaf surface. This test represents the approximate concentration of virus particles in a standard extract of a ½-inch disk of a mosaic air-cured leaf.

a sterile piece of cheesecloth. The three leaves showing the highest number of local lesions were used throughout as the basis for determining the amount of virus surviving in the sample.

Aside from the method of determining the amount of virus present, the technique used for the different experiments has varied greatly and may best be noted briefly in connection with the individual experiments.

EXPERIMENTAL RESULTS

THE COURSE OF DEVELOPMENT OF THE DISEASE

It is essential to an understanding of the factors influencing disease occurrence to become familiar with the natural and normal progress of the disease in the field during the season, as well as with its behavior in successive years. Tobacco mosaic is characteristically not of the epidemic type, chiefly because its occurrence is not largely dependent upon somewhat prolonged favorable weather conditions, nor upon insect agents of dissemination. Mosaic development in the crop is dependent rather upon the presence of infectious material, and certain momentary circumstances favoring infection. Many factors of environment do, however, influence the survival of the virus, infection, and dissemination, as will be shown, but, in general, this environment is more regular from year to year than is that of certain other disease-influencing factors. Tobacco mosaic is, therefore, never conspicuous by its absence in a growing section, but, on the other hand, it has rarely been seriously destructive over a wide area. Infection normally creeps into the field gradually throughout the season, and neighboring fields on the same or different farms may vary greatly in the percentage of mosaic.

Surveys of individual fields during the season clearly establish the normal course of mosaic development, except in those comparatively rare instances where the crop is transplanted with large percentages of infected seedlings. The percentage of mosaic increase, above that due to transplanting infected plants, is at first low, but expands gradually throughout the season if opportunity for infection is present (table 1). Additional data of this nature may be found in an earlier report of field studies (*9*) and in surveys by Wolf (*14*).

Equally pertinent is the relation between the amount of mosaic on previous crops and the percentage of infection on succeeding crops, as was shown by data compiled at this station in 1929 (*9*), which has since been repeatedly verified (table 2).

TABLE 1.—*Increase of tobacco mosaic during the growing season and from year to year following repeated culture to tobacco on 2 fields on East Hill Farm, Madison, Wis.*

Crop year	Field 6						Field 8				
	Previous crops	Plants	Mosaic—				Previous crops	Plants	Mosaic—		
			After 1 month	At topping stage	At harvest stage	In second growth			After 1 month	At topping stage	At harvest stage
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Percent*	*Percent*
1927	0	6,500	0.2	.....	1.5	.....	.....	.....	.....	.....	.....
1928	1	6,630	.3	1.9	4.6	.....	.....	.....	.....	.....	.....
1929	2	6,000	.....	2.6	.....	.....	.....	.....	.....	.....	.....
1930	3	5,945	.7	.....	1.5	.....	0	7,625	.....	0.1	0.3
1931	4	2,646	.8	4.0	45.0	.....	1	5,258	0.1	1.2	8.5
1932	5	2,890	3.1	12.0	36.0	.....	2	5,524	.1	1.5	5.0
1933	6	2,813	.4	15.7	29.7	99.1	3	5,714	.2	2.6	5.6
1934	7	2,883	2.4	6.0	13.8	95.0	4	2,923	.4	3.3	18.9
1935	8	2,975	4.1	8.4	12.8	73.3	5	4,960	.2	4.7	11.5
1936 [1]	9	2,813	.6	.9	1.8	.....	6	4,262	.01	.2	.8

[1] Season of unusual desiccation of surface soil.

TABLE 2.—*Comparison of percentage and rate of increase of tobacco mosaic on infested and on uninfested soil in different years, plants from the same seedbeds being used each year*

Year	Mosaic in infested soil—			Mosaic in uninfested soil—		
	After 1 month	At topping stage	At harvest stage	After 1 month	At topping stage	At harvest stage
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1926	4.5	13.8		0.2	0.6	
1927	2.4	16.8	46.1	.2		1.5
1928		8.3	32.1	.3	1.9	4.6
1929	3.5	21.2		1.3	[1] 10.3	
1930	6.9		56.4	.07		.3
1931	4.3	25.0	83.0	.07	1.2	8.5
1932	5.2	17.0	66.0	.07	1.5	5.0
Average	4.5	17.0	56.7	.3	3.1	4.0

[1] Reason for high count unknown, but considerable cucumber mosaic was found in this field in a later year.

If diseased seedlings are transplanted or if these seedlings are infected at the time of transplanting, such plants will be the first to show field symptoms, though signs of mosaic may be delayed as long as 3 to 4 weeks in unfavorable weather. Infection from the seedbeds is usually either characteristically localized or distributed, but this may also be true of infection originating at the time of transplanting, though more often the latter is scattered. Following these early infections, which may commonly be less than 1 percent, the disease either makes but little further progress until late in the season, or a gradual percentage increase occurs throughout the season, which may reach 25, 50, or even 100 percent on the mature crop or second growth. It is evident from field records that high percentages of infection may take place after planting and before topping operations (tables 1 and 2), and that, while many of these infections evidently take place gradually, others may develop in fairly distinct waves. It is on these new field infections, which are not clearly attributable to transmission by cultural operations, that special interest is centered. The explanation must be sought in the manner of survival of the virus from year to year, and in the means of infection and dissemination.

SURVIVAL OF THE VIRUS IN HARVESTED TOBACCO

The ordinary tobacco mosaic virus (*tobacco virus 1*) is unusual not only as compared to the numerous other plant viruses, but as compared to most other plant pathogens, in that it can survive for extraordinarily long periods in dead plant tissues when such tissues are maintained in a dry state or otherwise protected from decomposition. Some overwintering may occur in perennial hosts, or in tobacco or other annuals where killing frosts do not occur, but this potential source of infection is no longer regarded as of much importance by most investigators. The problem of survival is, therefore, rather one of the relation of conditions to the inactivation of the virus in the dead infested host tissues. For practical purposes, it is desirable to separate the survival studies into two major groups, namely, (1) that which occurs in harvested tobacco, including all plant parts removed from the field, and (2) that which occurs in plant parts remaining in

the field. The environment to which these groups of plant tissues are subjected is naturally quite different. In addition, wide variations may exist in the environment to which subdivisions of these groups are exposed, and these will be considered separately as far as seems profitable in a single contribution on the subject.

Influence of Curing

In a previous publication from this laboratory (*9*), limited data were presented indicating that the virus may become inactivated during the air-curing process after a period of 10 weeks. The newer trials do not support the conclusion that such inactivation is at all common or normal. The earlier results may be explained on the basis that the leaves selected for the trials were infected with the short-lived cucumber mosaic virus, a disease that was not recognized as occurring on field tobacco until some years later (*6*). It is also possible that the leaves selected for the test were actually infected with *tobacco virus 1* but exposed in such a position in the curing shed as to permit sufficient decomposition to inactivate the virus without serious injury to the leaf. A considerable amount of scattered evidence has been secured in the present study which shows that the virus may occasionally be inactivated in cured-leaf and other tissues through "natural" means without seriously damaging the structure of the tissue.

However this may be, repeated trials with known tobacco mosaic leaves, tested quantitatively by the disk (cork-borer samples of like area) method before, during, and after curing, under different conditions of temperature and humidity have failed on the average to show any appreciable loss of virus concentration as a consequence of the curing process (table 3), and it may be assumed for all practical purposes that air curing does not materially inactivate the virus of ordinary tobacco mosaic (fig. 1).

Table 3.—*Influence of air curing under different environmental conditions upon the survival of the virus*

[Samples ¾-inch disks, ground up with 2.5 cc of water and inoculated to hybrid host]

Place cured	Approximate average temperature	Approximate average relative humidity	Before curing		After air curing	
			Leaves sampled	Average number of lesions to sample	Leaves sampled	Average number of lesions to sample
	° *F.*	*Percent*	*Number*		*Number*	
Curing shed (lot 1)	70	75	3	553	3	345
Curing shed (lot 2)	70	75	3	633	9	777
Cellar	60	90	6	933	6	1,183
Air-control chamber	100	95	6	750	6	358
Basement room	70	50	6	823	6	850
Total or average			24	738	30	703

The situation in flue-cured tobacco may be expected to be quite different. During the process the tobacco may be gradually raised to a temperature of 180° F. (82° C.) or even higher and maintained at such temperatures for 12 to 24 hours. No extensive determinations have been made so far as the writer knows on the actual survival in

flue curing, but, according to Busch and Wolf (*1*), the virus is usually destroyed. The thermal death point of the virus in dry leaves over long periods of exposure at temperatures below about 90° C. (194° F.) is in some doubt. It is now generally agreed that its maximum limit in extract is close to 93° C. for 10 minutes. Price (*12*) reports inactivation in 13 hours at 80° C., in extract diluted 1–20 with water, and this determination appears according to the writer's data to be somewhat below the required exposure for inactivation in dry tissue.

The method used for determining the thermal death point over long exposures in the present experiments consisted in cutting uniform disks of green mosaic leaves with a cork borer, placing four to six disks in each Petri dish on filter paper, and drying at 50° C. (122° F.) for 24 hours. The dishes were then transferred to a constant-temperature oven, where they were heated to different temperatures for different lengths of time. The disks were then ground up with 5 cc of water and inoculated to the hybrid test plants, and the number of local lesions developed was recorded. The results have been summarized and grouped into classes in table 4. The results show that a temperature of 83° to 85° C. (181° to 185° F.) for at least 24 hours may be required to inactivate completely all the virus in dry-leaf tissue. This exposure is apparently at about the average temperature and time required for completing the flue-curing process. When the practical difficulty of assuring the maintenance of such killing temperatures in all portions of the curing barn for a period of 12 to 24 hours is taken into consideration, it seems extremely doubtful that flue curing can be relied upon to completely inactivate the virus. The conclusion therefore seems justified that even flue-cured tobacco should be regarded as a potential source of infection, and this idea has been verified further by finding the virus in random samples of flue-cured leaf tobacco secured from the flue-cured districts.

TABLE 4.—*Influence of higher temperatures over long periods of exposure, as in flue curing, on the inactivation of the virus*

[Sample ½-inch disks, ground up with 5 cc of water and inoculated to hybrid host]

Temperature	Exposed 1 to 4 hours		Exposed 8 to 12 hours		Exposed 12 to 24 hours		Exposed 24 to 48 hours	
	Tests	Average number of lesions	Tests	Average number of lesions	Tests	Average number of lesions	Tests	Average number of lesions
	Number		*Number*		*Number*		*Number*	
30° C. (86° F.)					1	1,200	3	1,300
50° C. (122° F.)					1	1,300	2	1,050
72° C. (162° F.)			2	475	3	300	2	10
78° C. (172° F.)	4	265	4	183	3	86	5	8
81° C. (178° F.)	4	637	5	231	8	67	4	1
84° C. (183° F.)	5	378	5	6	3	18	4	0

INFLUENCE OF STORAGE AND MANUFACTURING

A limited amount of inactivation of the virus appears to occur under practical storage conditions where the tobacco is sufficiently moist to permit some microbial activity. The amount of inactivation resulting directly from fermentation has not been investigated. It is clear, however, from the data on survival in manufactured tobacco,

that neither the normal storage period nor fermentation under average conditions influences the virus concentration sufficiently to be of practical interest in the present connection.

The presence of the virus in commercial brands of tobacco (especially chewing, pipe, and cigarette) has been reported, especially by Valleau and Johnson (*13*), Fukushi (*3*), and Busch and Wolf (*1*). These reports give little or no information relative to the concentration or amount of virus present, the tests having been performed by the older method of inoculation. A reexamination of this subject by the local-lesion method is therefore of some interest, since it may be assumed that the potential danger of field infection from this source depends in considerable measure upon the virus concentration present. The summarized results of these tests are shown in table 5. The results are not in complete agreement with the results reported by others, in that commercial chewing brands were found to be least infectious, no infection having been secured from 10 different brands. Pipe tobacco also was found to be low in virus concentration, but a surprisingly high concentration was found in certain brands of cigars and cigarettes (fig. 2). These commercial brands were selected at random from different stores and at different times of the year, usually only one sample being taken from a box, carton, or package. It was interesting to note the consistency of most popular brands with respect to virus concentration, some running regularly low, others uniformly high.

TABLE 5.—*Frequency and concentration of the virus in random samples of Wisconsin leaf tobacco and in commercial brands of tobacco*

[Samples 1 to 3 g ground up with 5 to 10 cc of water and inoculated to hybrid host]

Form	Brands sampled	Frequency of virus			Concentration of virus	
		Samples	Samples infested		Range of lesions	Average number of lesions
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*
Leaf tobacco	[1] 100	100	30	30	0-1,000	79
Cigars	30	61	41	67	0-800	55
Cigarettes	12	54	44	81	0-480	49
Pipe smoking	8	21	13	62	0-27	3
Chewing and snuff	10	13	0	0	0	0

[1] Different farms in this case.

The absence or low concentration of the virus in most brands of chewing tobacco, snuff, and pipe smoking must be largely attributed to the heat applied in processing and manufacturing. Its common presence in cigars is to be expected since little or no "extra" heat is used on cigar tobacco. It is possible that the amount of heat employed in cigarette manufacture varies sufficiently to determine the degree of inactivation of the virus, and that the type and percentage of tobacco used may also be a factor. The uniform blending results in a fairly constant virus content, so that, whereas one popular brand averaged no more than 3 lesions per cigarette, another popular brand averaged 200 lesions per cigarette.

Comparable random samples of leaf tobacco from 100 different Wisconsin farms yielded 30 infectious samples, indicating the per-

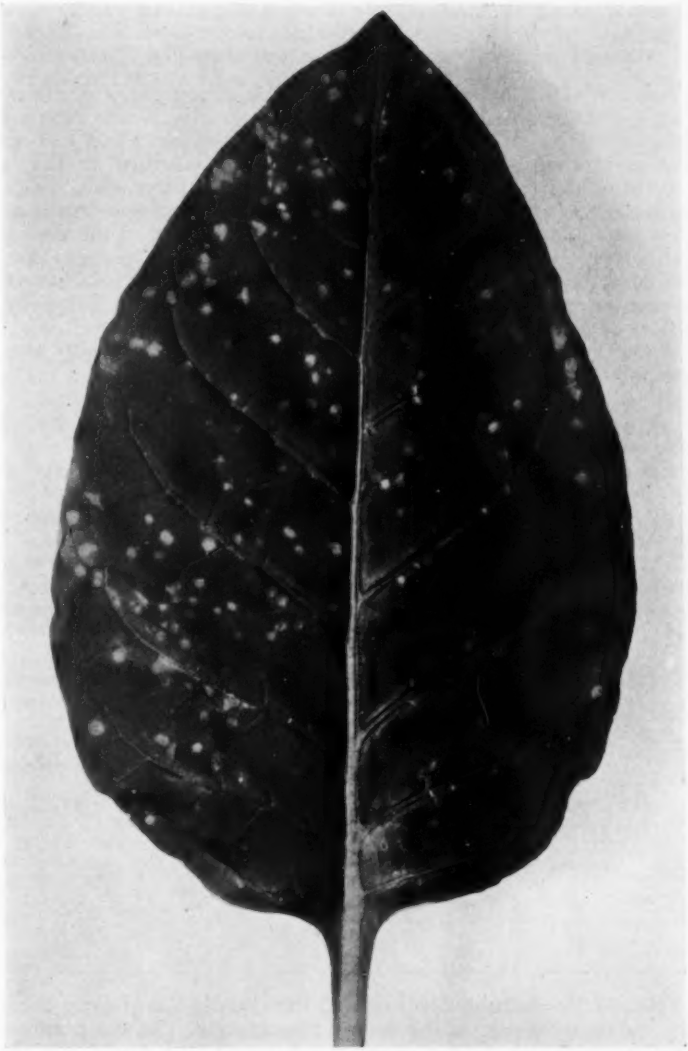

FIGURE 2.—Local lesions resulting from wiping the hybrid host with an extract from one cigarette of a popular brand. Other brands contain much lower concentrations of virus.

centage of tobacco affected with mosaic in this State. The use of such tobacco for home consumption is no doubt a more important potential source of infection in some growing districts than in others.

It is clear, however, that since such leaf tobacco is not usually blended (mixed) in quantities, it is considerably less likely to contain virus than are commercial brands (table 5). The relation of these tobaccos to crop infection is discussed in a later section of this paper.

Survival in Refuse and Byproducts

Tobacco stalks and leaf waste from the curing sheds or stripping rooms, and to some extent the seed chaff, constitute the farm refuse from harvested tobacco. The leaf midrib ("stems") and leaf waste from stemmeries, warehouses, and factories, returned to the farm directly or indirectly for fertilizer or insecticidal purposes, make up further refuse which bears consideration as a potential source of virus infection. These materials are usually protected from decay or weathering for long periods and present quite another problem to that of refuse remaining in the field. If the refuse from harvested tobacco is applied to the land in the autumn, it may naturally be subject to the same virus-inactivating influences as field refuse.

When the farm or factory refuse is maintained in essentially as good storage conditions as the leaf tobacco, it may be surmised that the virus will also survive in the refuse for a corresponding time, i. e., for several years. The actual environmental conditions are, however, quite variable, even when the various plant tissues are in close association, as in curing, storage, and processing. The stalks and midribs, being more retentive of moisture, are sometimes more subject to decay than is the leaf web, and it has consequently been found that the virus often does not survive as readily in these tissues as in the leaf web. If maintained under very dry conditions, the virus may survive quite as well in one tissue as in another (table 6), though the concentration of the virus in the different tissues may vary considerably.

Table 6.—*Survival of tobacco virus 1 in infested plant tissues stored in air-dry condition at room temperatures for different lengths of time*

Age (months)	Lesions on 3 hybrid leaves from—					Age (months)	Lesions on 3 hybrid leaves from—				
	Roots	Stubble wood	Stalks	Sucker stems	Leaf web		Roots	Stubble wood	Stalks	Sucker stems	Leaf web
	Number	*Number*	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*	*Number*
0	54	35	64	3	635	9	53	35	12	72	1,000
1	687	744	199	820	1,270	14	600	------	400	600	1,400
2	265	508	350	520	1,000	17	160	------	26	300	900
3	246	312	54	285	554	20	300	82	96	800	1,200
6	190	37	38	270	1,200						

Freezing, or alternate freezing and thawing, in the absence of moisture has no influence on the rate of inactivation. In the presence of moisture, inactivation may go on at a comparatively rapid rate under conditions of daily alternate freezing and thawing (table 7). That inactivation in moist tissues is largely if not entirely due to microbial action, has been shown by the growth of pure cultures of many fungi and bacteria on virus-infested aseptic (by heat) leaf disks in nutrient broth and on nutrient agar. Although the efficiency of the micro-

organisms as inactivators apparently varies greatly, a considerable number of the common soil organisms are capable of inactivating the virus.

Tobacco refuse from the farm, such as stalks and leaf waste, is frequently left over winter in curing sheds or it may be stacked out of doors to a height sufficient to afford protection from moisture to a large part of the refuse. This barn refuse is, therefore, not to be regarded as free from the virus (table 8) until it has weathered for a considerable time or is largely decomposed. Stalks and other refuse spread on the land in the early winter are usually inactivated by late spring.

TABLE 7.—*Influence of different environmental conditions on the rate of inactivation of the virus*

[Three ¾-inch air-cured disks of infested leaf web in cheesecloth-covered vials, ground up with 5 cc of water and inoculated to hybrid host]

Month	Weather		Lesions [1] on 3 hybrid leaves from exposures to—						
	Average monthly temperature	Snowfall	Outdoors continuous; sheltered	Outdoors 24 hours; moist chamber 24 hours	Moist chamber 26° C., continuous	Refrigerator −5° C., continuous	Refrigerator 24 hours; room 24 hours	Dry room 22° C., continuous	Outdoors under snow until Mar. 15
	°F.	*Inches*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Started Jan. 16, 1936				1,200	1,200			1,100	1,200
January	11	16.8	500	700		300	700	650	
February	6	17.9	650	265	177	800	750	775	
March	35	2.4	950	400	48	800	1,200	900	1,000
April	41	7.3	800	14	0	700	800	700	850
May		0	800	0	0	900	900	900	700

[1] Figures in round numbers estimated.

TABLE 8.—*Survival of tobacco virus 1 in plant tissues stored outdoors, but protected from direct precipitation and from absorption of moisture from the soil, under conditions similar to those in average air-curing shed*

Month	Weather during preceding period				Lesions on 3 hybrid leaves from—			
	Temperature		Relative humidity		Roots	Stalks	Stipules	Pods
	Average minimum	Average maximum	Average minimum	Average maximum				
	°F.	°F.	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Number*
August 1934					1,200	547		
February 1935	8	69	60	93	172	406	304	268
June 1935	28	71	59	87	9	550	440	112
November 1935	28	85	61	89	22	62	85	43
January 1936	4	26	84	93	105	99	179	20
March 1936	−3	43	67	92	46	27	96	37
April 1936	33	50	60	77	124	44	6	46
May 1936	53	74	52	72	167	160	108	30

Tobacco byproducts from stemmeries, warehouses, or factories, which are usually returned to the farm in the form of fertilizers or insecticides, should be regarded as potential sources of infection. However, the processing applied to some types of leaf tobacco destroys the virus, as in the case of the artificial heating to which stemming

tobacco may be subjected in the larger concerns. On the other hand, stems directly from infected cigar tobacco are almost certain to contain the virus unless subsequently stored for considerable periods under moist conditions, or purposely inactivated by heat. Unless the source and treatment of the stems is definitely known, it is advisable to regard them as infectious. The virus may, however, become inactivated at a surprisingly rapid rate when the stems are lying upon the soil surface exposed to weathering. It seems likely that the point of inactivation may be closely approached in these tissues, then halted by desiccation; and when favorable conditions for inactivation again develop, the process may be rapidly completed.

Nicotine extracts for insecticidal purposes, usually extracted with heat, naturally are free from virus. Tobacco dust or powder from different sources has been found to contain both low and high concentrations of virus, but since these are not commonly applied directly to tobacco plants, the danger of infection from this source is small. Although the virus does not enter the seed, and hence is not transmitted to the succeeding crop in this manner, the seed pods themselves may contain the virus (table 8), and if such infested chaff is not removed before sowing, considerable amounts of virus may be introduced directly into the seedbeds.

SURVIVAL OF THE VIRUS IN FIELD REFUSE

INFLUENCE OF WEATHERING

Roots, stubble, tops, suckers, leaf waste, and stalks in the case of primed tobacco, remaining in the soil or on the soil surface over winter, are potential sources of infection. It has already been shown that the virus may remain alive over winter in these tissues when under the soil surface (*5*) and hence protected from direct weathering. A large percentage of this material may remain on the soil surface itself unless plowed under, and even when fall-plowed, roots or other plant parts may be left exposed on the surface. The virus in plant parts exposed to direct weathering may conceivably survive for longer or shorter periods of time than similar tissues underneath the soil surface. The particular weather conditions obtaining may also greatly influence the survival. It has already been shown that freezing alone or freezing and thawing as such does not appreciably inactivate the virus in plant tissues, but that, on the other hand, a continued frozen condition tends to preserve the virus. It may consequently be expected that the virus will survive for longer periods in cold than in warm winter climates. Dry weather, which retards decay, aids in preserving the virus in tissues, whereas a covering of snow, serving to maintain a moisture supply as well as protection from freezing, may hasten decay inactivation in tissues. On the other hand, drying of tissues already partly decayed, such as moist roots overwintered in the soil, tends to hasten or complete the inactivation. Some of the data secured, bearing on the influence of weathering, are presented in table 9. The results show that inactivation was somewhat more rapid below snow than above the surface of the snow, under conditions of continued cold weather. However, it is clear from a comparison of the roots in tables 9 and 10, that the virus usually survives for longer periods and at higher concen-

trations beneath the soil surface than in the same tissues on the soil surface. Aside from the direct action of microbial decay, there seem to be other factors of "weathering" (perhaps oxidation or adsorption) which operate, hastening the inactivation in tissues exposed directly to the atmosphere. This is illustrated by the frequent observation that the virus in the fairly well preserved tissues of stalks and stubble of plants standing in the field over winter may be entirely inactivated, whereas the virus is present in high concentrations in the partially decayed roots of the same plant (fig. 3).

TABLE 9.—*Influence of weathering on the inactivation of the virus in plant tissues above the soil-surface, with and without snow covering, started Oct. 1, 1935*

KEPT ABOVE SNOW SURFACE

Month tested	Weather [1]		Lesions on 3 leaves of hybrid, from—						
	Average monthly temperature	Snowfall	Roots	Stalks	Stipules	Sucker stems	Leaf web	Leaf stems	Seed pods
	°F.	*Inches*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
November	34	1.4	108	309	22	----------	431	----------	56
December	21	6.7	250	295	500	----------	875	----------	24
January	11	16.8	54	89	16	----------	19	----------	4
February	6	17.9	34	50	1	3	8	----------	3
March	35	2.4	91	310	130	----------	65	400	0
April	41	7.3	25	23	----------	----------	4	1	0
May	64.0	0	6	135	0	3	0	0	0
June	----------	----------	30	123	----------	----------	0	----------	----------
BELOW SNOW [2] UNTIL APRIL 1936									
Mar. 23	35	2.4	30	37	----------	183	87	96	----------
April	41	7.3	47	0	0	0	1	----------	0
May	64.0	0	18	0	0	0	0	0	0
June	----------	----------	0	0	----------	0	----------	----------	----------

[1] Weather Bureau records, Madison, Wis.
[2] See above for snowfall November to March.

TABLE 10.—*Overwintering of the virus in different tobacco tissues and in soil collected from different Wisconsin fields in 1935 and 1936, as measured by the local-lesion method*

Field location	Date	Roots		Stubble wood		Stubble bark		Stalk		Soil	
		Samples	Average number of lesions	Samples	Average number of lesions	Samples	Average number of lesions	Samples	Average number of lesions	Samples	Average number of lesions
		Number		*Number*		*Number*		*Number*		*Number*	
Madison	Apr. 8 and June 5, 1935.	9	740	9	83	5	393	7	10	13	27
Sun Prairie	June 1 and June 11, 1935.	6	39	6	1	----------	----------	1	0	6	5
Edgerton	June 3 and June 12, 1935.	16	258	1	33	3	143	3	1	6	37
Madison	Apr. 14 and Apr. 29, 1936.	5	436	----------	----------	----------	----------	4	4	4	28
Edgerton	Apr. 21, 1936	7	126	3	10	3	19	----------	----------	10	11
Madison	June 2, 1936	5	189	4	1	4	2	5	0	4	[1] 9
Edgerton	June 1, 1936	6	244	6	3	4	43	----------	----------	4	15

[1] Soil relatively dry. Total rainfall for May, 0.79 inch.

FIGURE 3.—Concentration of virus overwintering in roots under practical field conditions. The lesions shown here represent only about one hundredth of those obtainable from the half-decayed roots of one plant.

The destructive action of weathering is rarely if ever sufficient to inactivate all the virus present in all the refuse from preceding crops, although it is generally true that the virus in the refuse on the soil surface is inactivated. That such overwintering of virus occurs in plant parts, especially roots, under field conditions has been reported previously (*9*) but the data presented show little relative to the concentration of virus particles present. Table 10 gives the number of local lesions obtained from various samples of decaying tissues from the preceding year's crop collected from tobacco fields as late as early June in 1935 and 1936, at which time transplanting of the new crop is under way. It is clear that these tissues contain sufficient virus not only to cause direct infection to the new crop from the tissues themselves, if conditions for infection are favorable, but also to thoroughly contaminate the soil through leaching of virus out of infested tissues.

SURVIVAL IN THE SOIL

The many phases involved in the behavior of the tobacco mosaic virus in the soil made it seem advisable to treat this subject in some detail in a separate paper (*5*). It was shown that the virus may come into intimate contact with the soil through leaching from virus-infested tissues, and that the virus could remain active in the soil in this form for as long as 1 year provided the soil was not thoroughly dried or frozen (fig. 4), both processes being very effective inactivators of virus extract in soil. The soil type did not exercise any major influence on the survival of the virus in the soil, although it was inactivated somewhat more slowly in some soils than in others in the absence of freezing and drying. For some reason not fully understood, the rate of inactivation was distinctly more rapid in pure sand than in field soils.

TABLE 11.—*Influence of different percentages of field soils on the rate of inactivation of the virus in sand*

[2 kg of sand or soil with 125 cc of full-strength virus extract, kept moist in covered pans stored in greenhouse, sampled by cork-borer method]

Amount soil added to sand (percent)	Lesions [1] on 3 hybrid leaves after virus being in—											
	West Virginia soil and sand						New York State soil and sand					
	0 week	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	0 week	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	1,000	750	235	121	55	55	1,200	900	482	161	19	12
1	1,100	1,200	282	172	65	10	1,250	1,000	424	169	144	125
5	1,200	1,300	1,200	192	800	244	1,400	1,000	900	400	181	133
10	1,300	1,300	1,100	269	600	900	1,300	1,100	1,200	500	500	400
25	1,200	1,400	1,300	600	1,000	400	1,300	1,300	1,500	600	600	500
50	1,100	1,500	1,400	1,100	700	1,300	1,450	1,400	1,500	600	600	600
75	1,000	1,600	1,400	900	500	1,200	1,200	1,400	1,500	600	400	500
100	1,000	1,600	1,400	1,100	600	1,400	1,150	1,300	1,400	600	700	600

[1] Round numbers estimated.

It has not been possible at this time to investigate in detail the unusual behavior of the virus in sand. It is of interest, however, to note the behavior of the virus in sand to which various percentages of the more virus-retentive field soils were added (table 11). Unfor-

tunately an extremely heavy inoculum was used in this experiment, resulting in a slower rate of inactivation, but it is evident that the

FIGURE 4.—Free virus survives readily in moist soil, protected from freezing. Lesions secured from 1 g of soil 4 months after mixing 1 part of virus extract with 20 parts of soil.

addition of as little as 5 percent of field soil to the sand markedly decreased the rate of inactivation. Since the most sandy field soils carry no more than 90 percent of sand, it may hardly be expected

that the physical nature of field soils plays a significant role in the survival of the virus. Insofar as this character influences rate of drying and aeration of soils, it may, however, influence virus inactivation in certain seasons.

Continued observations have been made on the time of survival of the virus in close association with the soil (i. e., as virus extract in soil) in the absence of drying or freezing. In the previous report (*5*) it was shown that the virus survived in this manner for a period of 12 months. Tests on some of these soils have continued during the past year, so that it can now be said that the virus may survive in soils for 24 months, although the percentage of virus particles surviving is, to be sure, relatively low (table 12). It was furthermore found that despite a tendency of the virus to become attenuated following continued association with soil, the large majority of the virus particles retained their original virulence.

TABLE 12.—*Persistence of tobacco virus 1 in soil, to which it was added in the form of extract, with the soils protected from desiccation and freezing*

Time (months)	Lesions obtained from soil type indicated						
	Merrimac sandy loam	Sandy loam (Wis.)	Miami silt loam	Collington fine sandy loam	Hagerstown loam	Huntington silt loam	Durham sandy loam
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	275	600	66	550	923	850	207
3	92	310	66	441	85	261	28
6	77	230	155	96	25	147	----
9	10	33	162	62	58	178	12
12	25	25	73	33	48	45	0
15	12	27	----	6	6	52	4
18	23	89	32	44	17	----	----
21	4	37	----	----	----	----	----
24	18	52	----	----	----	----	----

Some significance may be attached to this observation in relation to the survival of the virus in the deeper layers of soil under field conditions, particularly where aeration is low, and where the virus may escape freezing and desiccation. It may frequently be true that holding tobacco or other susceptible hosts off the land for one season may largely eliminate the danger of infection from the soil, but it is clear from these results, and from the known persistence of the virus in protected plant tissues for long periods, that "resting" the land from tobacco for one season may not always be sufficient to assure complete freedom of the soil from the virus (*14*). Fall plowing and attempts to reduce the soil infestation by the removal of the tobacco stubble, together with the main roots and second growth, during the 1931 and 1934 seasons failed to reduce the amount of infection sufficiently under Wisconsin conditions to warrant recommending these practices as control measures. In the light of our knowledge of the survival of the virus in the soil and roots, it is furthermore difficult to see how these practices could be very efficacious in fields where high percentages of infection exist. Wolf (*14*) and Lehman (*10*), however, report more favorable results from removal of stubble and fall plowing under North Carolina conditions.

SURVIVAL ON FARM EQUIPMENT

There is some probability that tobacco farm equipment, such as seedbed cloth and lumber, plant boxes, tools, and machinery, may harbor sufficient virus under practical conditions to initiate a small percentage of seedbed or field infection. This may occur as a result of small pieces of virus-infested plant tissue remaining on such equipment from one growing season to another. In such instances the storage conditions of the equipment may be expected to influence the amount of survival of virus. Special consideration has not been given to this problem in the present investigation, but the same principles discussed as applying to infested refuse and soil may be expected to apply to contaminated equipment. Soil on equipment either under out-of-door or indoor storage may consequently be expected to dry or freeze sufficiently to inactivate any virus associated directly with it. Plant tissues on equipment may, however, be expected to carry the virus for long periods, even under outdoor storage exposed to weathering, as shown previously (*9*) where survival for as long as 2 years on seedbed cloth or frame boards was secured.

INFECTION AND DISSEMINATION

The studies on the survival of the virus which have been described are intended to define more clearly the potential sources of virus infection to the crop in the seedbed and in the field. The problems of infection and dissemination may or may not be closely related to all the potential sources of infection, and it is the purpose of this further investigation to test some of the hypotheses which are thereby presented with ordinary tobacco mosaic. The questions of infection and dissemination are often so closely associated that it does not appear profitable to discuss them separately. Tobacco mosaic is typically an infectious disease in the sense that dissemination may result only through direct contact with the infectious material. It is furthermore practically certain that infection can only develop through wounds, although these wounds need be only microscopic in size and not easily accounted for.

It is now fairly well established that *tobacco virus 1* is not typically transmitted by aphids or other insects in tobacco fields, although certain other less common viruses on tobacco, such as cucumber mosaic, are commonly disseminated in this manner (*4*).

THE POINT OF INFECTION

Although it has been stated that infection may occur only through wounds, it does not follow that the virus may enter the host through all organs of the plant, even though such organs are susceptible to it. Since the relation of soil infestation to the development of mosaic has been frequently shown, it might be assumed that infection frequently occurs through wounded roots. Repeated attempts, however, to secure infection in this manner by various methods have failed or left grave doubt as to the actual point at which infection developed. Other workers have reported similar results (*10, 11*).

Perhaps the simplest way to demonstrate the resistance of the roots to infection under greenhouse conditions is to inoculate a portion of the soil heavily with virus extract, placing a ½- to 1-inch layer of

virus-free soil on the surface. Sowing or transplanting may then be done with little danger of early stem or leaf infection. Little or no infection may result, and the roots may then be cut or mutilated underground without inoculating the leaves, if some caution is practiced. If the original inoculation is heavy, some infection may result, especially in transplanted plants (table 13), possibly because of virus moving upward in the soil with the water, but a surprisingly low percentage of infection may be maintained for a long period in heavily infested soil if adequate protection of leaf and stem is given. More reliable and convincing evidence has been secured by inoculating the roots directly with the virus. The exposed roots were produced by placing the potted plants over water in earthenware crocks of suitable size, hence permitting the roots to grow out of the base of the pot into the dark and moist chamber below. By using various methods of inoculation of the free roots, it has been found that not only do the leaves fail to develop symptoms, but the roots themselves generally fail to become infected locally, as determined by extraction and reinoculation to the hybrid by the local-lesion method. Likewise inoculation of the roots of the hybrid fails to induce necrosis at the point of inoculation (a symptom which might be expected on this host), and the inoculated portions of the roots fail to yield appreciably more virus than was originally introduced (table 14). The data secured by this and other methods are not sufficient to show that infection may never occur through the roots, but they do plainly prove that such infection is rare.

TABLE 13.—*Relation of the position of the virus inoculum in the soil to the rate and percentage of infection of tobacco seedlings so planted as to avoid hand contamination*

Inoculum	Position of inoculum in soil	Plants infected out of 25 transplanted			
		After 12 days		After 35 days	
		Havana Seed	Hybrid	Havana Seed	Hybrid
		Number	*Number*	*Number*	*Number*
Dry granulated mosaic leaves	On surface	12	4	19	7
	1 inch below surface	0	0	1	0
Extract from green leaves	On surface	13	18	23	24
	1 inch below surface	0	0	3	3

TABLE 14.—*Results of attempts at direct inoculation of exposed roots with tobacco virus 1 by means of cutting 50 or more young roots per plant under virus inoculum and allowing roots to remain suspended in inoculum for periods from 10 minutes to 3 days, after which roots were washed and grown in several changes of water*

[Exposed roots grew through base of pots suspended in earthenware crocks]

Experiment no.	Concentration of inoculum	Lesions on 3 leaves	Roots inoculated		Plants diseased	Average number of lesions on 3 hybrid leaves from inoculated roots
			Variety	Plants		
		Number		*Number*	*Number*	
1	1–1,000	144	Hybrid	5	0	24
2	1–1,000	76	Havana Seed	5	0	0
3	1–1,000	76	Hybrid	4	0	8
4	1–100		Havana Seed	1	0	32
5	1–100		do	3	0	3
6	1–10		do	2	0	48
7	Controls, 5 leaf-inoculated plants				5	350

The leaves and the stem of the plant are, as is well known, highly susceptible to the virus by artificial inoculation. The size and number of wounds bear little relation to the percentage of plants infected when the virus is definitely introduced, but where the virus concentration is low or irregularly scattered, and exposure to it is only at random, the size and number of wounds (total surface) exposed may be closely correlated with the percentage of infection. For all practical purposes, it may be assumed that infection occurs through broken trichomes or hairs on the leaf or stem. When these come in contact with any object, such as the hands, clothes, soil, or equipment, they are easily broken in considerable numbers, and if the virus is present at the same point, infection is very likely to occur. Although actual infection is apparently almost immediate, several days or even weeks may elapse before the virus multiplies and spreads sufficiently to yield typical symptoms of disease. In young hybrid plants the spread of infection may be easily followed by the progress of the necrotic areas from the point of infection (*7*), and the point of infection may be quickly and easily determined. By this method it is readily shown that transplanted seedlings are often infected on the stem or on leaves below the soil surface, as well as on the leaves above the surface.

It has been claimed (*2*) that virus infection may occur through the stomata when the virus is present as in spray inoculation. Considerable doubt was cast upon this hypothesis in an earlier paper (*7*) from this laboratory. Further results by a water-soaking method of favoring infection tend further to disprove this idea. Leaves which are water-soaked by the water-pressure method (*8*) and evidently not actually wounded, though becoming extremely susceptible to stomatal infection and the progress of certain bacterial organisms, do not become increasingly susceptible to *tobacco virus 1* (table 15). Hence, if the virus enters the stomatal chamber it does not find the situation favorable for infection (fig. 5).

TABLE 15.—*Results of spraying virus on leaves previously water-soaked by means of a high water pressure on the root system of the plants*

Trial no.	Pretreatment of hybrid host	Concentration of spray	Lesions on 3 leaves of hybrid	
			Sprayed only [1]	Wiped after spray
			Number	*Number*
1	Water-soaked	1- 10	4	600
2	do	1- 10	9	600
3	do	1-100	8	198
4	do	1-100	3	174
5	do	1- 10	6	837
6	Not water-soaked	1- 10	4	600
7, 8, 9	do	1- 10	0	[2] 438

[1] Some leaves were probably touched lightly when spraying in the first 3 trials.
[2] Average.

INFECTION THROUGH THE USE OF TOBACCO IN CULTURAL OPERATIONS

The use of tobacco by growers during cultural operations is common, and the presence of the virus in relatively high concentrations in some lots of commercial and home-grown tobacco has been shown (table 5).

Dissemination through the use of chewing tobaccos is regarded as a factor of major importance by certain observers (*13*, *14*). Commer-

FIGURE 5.—A hybrid leaf which was first water-soaked by applying high water pressure to the root system. It was then sprayed with 1-10 virus extract, after which the left side of the leaf was wiped with cheesecloth. One local lesion developed on the right side, possibly, but not certainly, through a stomatal opening.

cial chewing tobaccos (plug, scrap, fine-cut, and snuff) are evidently least likely to carry the virus because of the high temperatures used

in processing and manufacturing. Leaf tobacco (twist, etc.), often used by growers in some districts, may contain large concentrations of virus, but not being blended, as in manufacturing, all lots are naturally less likely to be infested. Data based upon 100 farmers' lots and collected at random show that 30 percent may be infested in varying degrees, although this percentage may be somewhat higher in certain other tobacco districts.

Infection from these sources depends essentially upon the hands becoming contaminated either through contact with saliva or through handling the dry tobacco, the virus being subsequently introduced through slight wounds made in handling the plants. Experimentally this may be easily shown to occur where intentional contamination and infection are performed (table 16). Where no intentional effort is made, the amount of infection is much less. It is, of course, quite impossible to judge from such trials as to how much infection of this type actually results under practical conditions. According to the field surveys conducted by others (*13*), infection from this source is common, though it should be pointed out that surveys of this sort would need to be very carefully conducted in order to eliminate at the same time all other factors which may have been concerned in the infection.

TABLE 16.—*Intentional as compared with unintentional transmission of the virus to plants during the use of tobacco when transplanting to virus-free soil*

Experiment no.	Form of tobacco	Virus concentration (lesions on 3 hybrid leaves)	Transmission by hand	Variety of test plant	Plants transplanted	Plants infected	Total lesions on hybrid plants
		Number			*Number*	*Number*	*Number*
1	Cigar smoking	[1] 800	Intentional	Hybrid	50	0	0
	do	800	Unintentional	do	25	0	0
	Cigar chewing	800	Intentional	do	25	2	2
2	Cigar smoking	328	do	Havana Seed	42	6	
	Cigar chewing	328	do	Hybrid	21	4	4
3	Cigarette smoking	163	Intentional	do	25	4	4
	do	163	Unintentional	do	25	0	0
4	Leaf chewing	150	Intentional (with saliva).	Havana Seed	28	26	
	do	150	do	Hybrid	28	20	37
5	None	None	None	Havana Seed	50	0	
	do	None	do	Hybrid	50	0	0

[1] Cigar wrapper subsequently shown to be free from virus.

INFECTION FROM CROP REFUSE

The greater part of the refuse from harvested tobacco is eventually returned to the land, in which circumstance it may bear the same relation to infection as field refuse. It should be recalled, however, that on account of the "sheltering" of harvested refuse for at least a portion of the year, the survival of the virus is prolonged in direct proportion to the length of time of sheltering.

Approximately 5 to 6 months of weathering is required to inactivate the virus in fully exposed plant tissues, consequently any refuse applied to the seedbeds or the field in the spring is potentially a source of infection. Several experiments were made to determine the means by which infection from such sources may occur, the hybrid host

and the Havana-Seed variety being used as test plants. In view of the results of a similar nature presented in other sections of this paper, these tests need not be presented in the present connection. It is obvious, however, that in the seedbeds the wounding necessary to infection is likely to be done either by hand in weeding and pulling plants, by cloth covers pulled over the plants, or by storms. The young tender seedlings are especially readily wounded, and the presence of any virus is almost certain to result in some infection. Although refuse such as stalks and stems may be almost completely plowed under in the field, the handling of occasional pieces of such refuse as may become entangled with the machinery in transplanting is especially likely to result in infection. The possibilities of infection in this manner are even greater from the overwintering roots in the field, portions of which are often far enough below the surface to preserve the virus, and yet close enough to the surface to interfere with cultural operations in the new crop. Field observations suggest that many of the early field infections may be most easily accounted for in this manner.

Infection From the Soil

Although it may be admitted that significant amounts of direct infection with ordinary tobacco mosaic may arise from several other sources, it must be concluded that the soil is a very common source of primary infection. The reasons for this conclusion are as follows: (1) Previously infested fields regularly develop a considerably higher percentage of mosaic than do newer fields (table 2); (2) except for such mosaic as is plainly due to infection before or at the time of transplanting, the disease develops gradually in the field during the early growing season in the absence of other sources of infection and dissemination (table 1); and (3) the virus regularly persists in the field soil in sufficient quantity and for sufficient time to account for this infection (table 10).

The problems connected with infection from the soil are largely those of determining the various ways in which actual infection occurs, and finding the reason for the erratic behavior in percentages of infection which develop. These problems must be explained on the basis of the amount of virus originally present, the amount inactivated through decomposition, freezing, or desiccation (*5*), and the number of wounds of large or slight degree to which the plants are exposed during the growing season. That abundant and frequent wounding may result from cultural operations or wind and rain must be granted. It may be recalled that the plant stems and the lower leaves are always in contact with the soil, and that storms may temporarily greatly increase the leaf area in actual contact with it (fig. 6). Under practical conditions it seems most likely that the amount of infection is limited more by the absence of the virus at the point of wounding than by the frequency of wounds. The number of virus particles present per gram of soil may consequently be expected to determine in large measure the percentage of infection that will develop. Determinations made on naturally heavily infested field soils show that when the number of virus particles is as high as approximately 25 per gram of soil, heavy field infection may be expected. Less than five particles of virus per gram of soil may be considered a light infestation. If tobacco is planted in soils contain-

ing a very high concentration of particles, and care is taken not to infect the plants with soil-contaminated hands, a high percentage of infection is still obtained (table 17). This infection plainly results from direct contact of the plants with the soil, and a large part of it comes through the leaves in contact with the soil (table 18). If plants are transplanted with contaminated hands from virus-infested soil, the infection is greater (table 19) and earlier. The operation of transplanting by hand or machine in virus-infested field soil is believed, on the basis of these experiments and field observation, to account for a large percentage of the early infections in the field. The percentage of infection may be expected to vary with many conditions, such as the degree of desiccation and consequent inactivation previously reached by the soil surface (table 20).

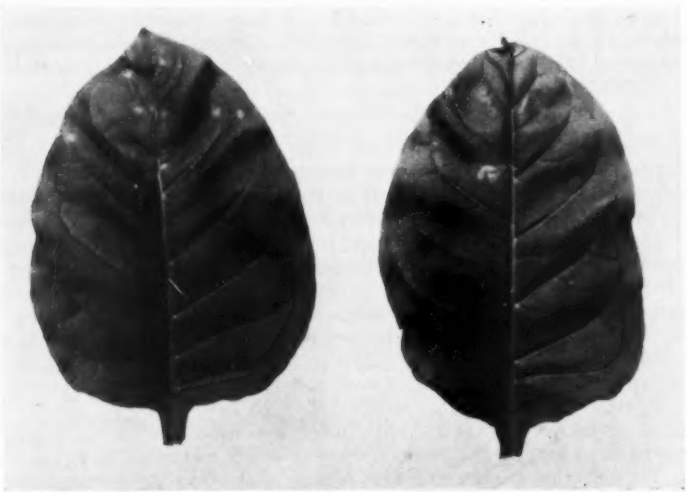

FIGURE 6.—Leaves of one hybrid plant dragged lightly for a distance of 2 feet over naturally infested overwintered field soil. Twelve points of infection with the virus may be noted on the twe leaves.

TABLE 17.—*Infection secured in transplanting tobacco to soils of various degrees of infestation under greenhouse conditions, special care being taken to avoid hand contamination from soil in planting*

Flat no.	Soil type	Lesions on 3 hybrid leaves from soil sample	Plants transplanted	Plants diseased	
		Number	*Number*	*Number*	*Percent*
1	Greenhouse compost	100+	50	35	70
2	do	100+	50	32	64
3	Silt loam	94	25	6	24
4	do	39	25	8	32
5	Sandy loam	37	25	10	40
6	Greenhouse compost	30	30	17	57
7	Sandy loam	21	50	41	82
8	Silt loam	18	100	88	88
9	do	16	50	25	50
10	Fine sandy loam	12	30	4	13
11	Silt loam	7	30	3	10
12	do	6	30	5	17
13	Greenhouse compost	0	50	0	0
14	do	0	50	0	0

TABLE 18.—*Results of preliminary experiments designed to illustrate direct virus infection through leaves in contact with infested soil, single leaves of hybrid plants in 4-inch pots having been placed temporarily in desired position on infested soil*

Experiment no.	Soil type	Lesions on 3 hybrid leaves from soil sample	Leaves lying on soil surface				Leaves dragged over soil surface	
			5 days, sprinkled only		10-minute air current			
			Number	Lesions	Number	Lesions	Number	Lesions
		Number		*Number*		*Number*		*Number*
1	Merrimac sandy loam	13	14	0			10	17
2	Durham sandy loam	700	10	16	10	151		
3	do	700	20	12	10	5		
4	Miami silt loam	39	10	1	10	0	21	134
5	do	39					18	47

TABLE 19.—*Amount of infection resulting from transplanting tobacco with hands contaminated with virus-infested soil, plants having been set in virus-free soil after handling moist infested soil*

Experiment no.	Data on source of hand contamination		Plants transplanted	Plants infected		Average number of lesions on 3 hybrid leaves
	Means of soil infestation	Concentration of virus, lesions on hybrid				
		Number	*Number*	*Number*	*Percent*	
1	Artificial	128	30	15	50	1
2	do	40	7	4	57	2
3	Natural	15	47	44	93	3
4	Artificial	128	30	20	66	
5	do	128	50	28	56	
6	do	128	30	30	100	
7	do	40	7	2	28	
8	Natural	7	75	9	12	
9	do	33	75	31	41	

TABLE 20.—*Relation between the vertical position of the virus in the soil and the rate of inactivation under conditions of gradual and uninterrupted loss of moisture from the soil surface*

[1 cork-borer set of samples taken from top down 1 inch, others from a horizontal position. Experiment started Jan. 21, approximately uniform at about 225 lesions per sample]

Level of sampling	Lesions on 3 hybrid leaves after—									
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	8 weeks	12 weeks	15 weeks	18 weeks
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Surface only	3	2	1	0	0					
Surface down 1 inch	274	105	23	12	7	25		0		
1 inch		62	106	115				0	0	
2 inches				26	27	50	12	1	0	0
3 inches		98	127	20	27		21	6	1	0
4 inches				17	21	46	45	6	12	0
5 inches		82	19	16	21		27	18	16	4
6 inches				18	26	48	30	10	7	13
8 inches		192	30	35	13			14	6	13
10 inches				71	24	56	54	5	6	10
12 inches	192		59	0	16		12	2	5	6

It is an interesting fact that tobacco may be sown in infested soil and grown to a considerable size in the absence of wounding without

infection resulting. Furthermore, such plants if pulled from the seedbed without allowing the hands to come in contact with the infested soil may be transplanted without resulting infection (table 21). When weeding and pulling plants under practical conditions, the hands naturally come in contact with some soil, and when this soil is infested, either as a result of previous cropping to tobacco or from harvested tobacco refuse, infection is almost certain to follow. Occasionally fairly heavy field infection is found which is plainly derived from apparently healthy seedbeds, some of which may be explained in this manner. Other infections may come about more indirectly from hand contamination through contact with occasional infected seedlings already present in the plant bed (table 22). Visible symptoms of mosaic are rare even on infected seedlings in seedbeds at the time of transplanting, owing either to recent infection or to the crowded growth of the plants. This absence of visible symptoms often accounts for the obscure origin of disease in uninfested field soils.

TABLE 21.—*Relation between tobacco seed sown in virus-infested soil and infection in the seedbed*

Soil inoculum used	Placement of inoculum	Lesions on 3 hybrid leaves from soil sample	Approximate plants exposed		Plants infected after 35 days	
			Havana Seed	Hybrid	Havana Seed	Hybrid
		Number	*Number*	*Number*	*Number*	*Number*
Dry, granulated mosaic leaves, 10 g.	Surface	40	100	75	[1] 0	0
	1 inch below surface	38	75	50	0	0
Extract from green leaves, 50 cc.	Surface	123	75	50	1	2
	1 inch below surface	200	50	50	0	0
Extract from green leaves, 100 cc.	Mixed	14+	500	500	[2] 0	[3] 0

[1] 42 transplants to pots without touching soil yielded no infection; 14 transplants to pots touching soil yielded 6 mosaic.
[2] 70 plants transplanted without precaution yielded 18 mosaic.
[3] 150 transplanted without precaution yielded 10 mosaic.

TABLE 22.—*Transmission of tobacco virus 1 by hand contamination when transplanting after having lightly touched mosaic seedlings*

Plants and condition of leaves	Total plants touched prior to planting 7 plants	Plants infected out of 7 planted	Lesions on hybrid plants		Plants and condition of leaves	Total plants touched prior to planting 7 plants	Plants infected out of 7 planted	Lesions on hybrid plants	
			Total	Average				Total	Average
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
Hybrid, leaves dry	1	7	22	3.1	Hybrid, leaves wet	1	4	7	1.7
	2	7	30	4.3		2	4	11	2.7
	3	7	40	5.7		3	5	11	2.2
	4	7	22	3.1		4	6	14	2.3
	5	7	23	3.3	Havana Seed, leaves wet	5	7	-------	-------
	6	5	8	1.6		6	7	-------	-------
	7	6	10	1.6		7	7	-------	-------
	7	4	8	2.0		8	7	-------	-------
	7	4	10	2.5		9	7	-------	-------
	7	6	11	1.8					
	7	4	7	1.7					

DISSEMINATION BY CULTURAL OPERATIONS

Transplants which are not infected at the time of transplanting may escape infection for varying periods in infested soil. If such plants could escape leaf and stem wounds from cultural operations or from

wind and storms, little further infection presumably would take place. The increase in infection between the transplanting and the topping operations appears to be particularly obscure in origin. Some investigators who are not convinced of the relation of direct soil infestation to this increase, are inclined to place special emphasis on direct transmission of the virus from mosaic to healthy plants through the operation of cultivating the crop (*10*). There is every reason to believe that such spread may and does occur, particularly as the plants grow larger and come regularly into contact with the men, horses, or equipment performing the work. Furthermore, it may be added that a considerable amount of dissemination through cultivation may result from direct transmission from infested soil to plants. By approximating cultivating conditions with soil-contaminated tools and clothes rubbed against hybrid plants, as many as 46 local lesions per plant have been secured on the leaves. Root wounding through cultivation in infested soils yielded no infection. The writer's experiments on the amount of dissemination, from either plant to plant or from soil to plant, directly traceable to cultivation, have not been sufficiently extensive to indicate the relative significance of this factor. The suggestion that much of this type of spread may be avoided by roguing out the mosaic plants has been made. It is not likely, however, that a reasonable amount of roguing will hold down the percentage of infection sufficiently on infested soils to greatly reduce such dissemination as may occur through cultivation.

That worming, topping, and suckering operations may result in extensive dissemination from mosaic plants to healthy plants has repeatedly been shown. This need not be discussed further, except to point out that, when hybrid plants have been topped with contaminated hands, local lesions have been observed at the point of infection (table 23). The amount of virus which can be transferred by the hand even after topping only one diseased plant is surprisingly large (fig. 7).

Suckering and harvesting also transmit large amounts of virus, and although this infection is normally too late to injure the crop, it often accounts for the general and rapid dissemination of the disease over the land and the increase from a moderate percentage of infection on the crop itself to as much as 100 percent on the secondary growth, which in turn builds up the soil infestation for the following year.

TABLE 23.—*Transmission of tobacco virus 1 by the topping practice as determined by the local-lesion method on the hybrid host*

Experiment no.	Mosaic plants previously topped	Hybrids topped successively	Hybrids infected by topping	Lesions on all hybrids topped	Average number of lesions on each hybrid topped	Experiment no.	Mosaic plants previously topped	Hybrids topped successively	Hybrids infected by topping	Lesions on all hybrids topped	Average number of lesions on each hybrid topped
	Number	*Number*	*Number*	*Number*			*Number*	*Number*	*Number*	*Number*	
1	1	5	4	7	1.7	3	2	6	4	7	1.1
	2	5	5	6	1.2		3	6	3	3	.5
	4	5	4	13	3.2		4	6	4	9	1.5
	8	5	5	37	7.4		5	6	6	16	2.7
	16	5	5	47	9.4						
2	1	5	4	13	3.2	Av.	4.2	5.3	4.5	15.4	3.1
	2	5	5	15	3.0						
	3	5	5	12	2.4						

DISSEMINATION BY STORMS

The relation of wind and rain, particularly in the form of heavy storms, to infection and dissemination of certain bacterial and fungus diseases of plants has long been known or suspected, although even here the details involved are in need of further study. Though it is not likely that storms have any important relation to infection and dissemination of plant virus diseases in general, the circumstances

FIGURE 7.—Local lesions on the upper leaves of a hybrid plant produced by topping the plant with a hand contaminated from topping a single mosaic plant.

with the ordinary tobacco mosaic disease are such that it is locigal to believe that dissemination by wind and rain should be concerned. With bacteria and fungi it is essential not only that the environment be made favorable for infection, but that a favorable condition be maintained for a considerable period of time. In the case of the tobacco virus infection is almost instantaneous once the wound and the virus occur at the same point. The wound may be extremely

slight and may consequently result from only gentle swaying of the plant, rubbing the leaf or stem against the soil surface or against a neighboring plant (table 24). Although water is not necessary for infection, as in the case of most fungi and bacteria, it may be a factor in producing wounds in the host and in carrying virus over the leaf surface. The heavier the storm, the greater the number of wounds which may be exposed to the chance presence of the virus. In this manner, some dissemination may occur from plant to plant or from soil to plant. It is not contended that the virus spreads for any distance in water or through the air in water particles, although this is not inconceivable since droplets of rain water on mosaic tobacco leaves or guttation water from mosaic tomato plants often contain the virus (table 25).

TABLE 24.—*Transmission of tobacco virus 1 from plant to plant when leaves are in contact, healthy hybrid plants having been alternated with mosaic-infested Havana-Seed plants under different conditions, 10 hybrid and mosaic-diseased leaves being in contact*

Experiment no.	Conditions (artificial) (1 hour)		Lesions on hybrid leaves	
	Wind	Moisture	Total	Average number
1	Moderate [1]	Dry	94	9.4
2	do.[1]	Wet	117	11.7
3	None	Dry	0	0
4	Strong [2]	Wet	29	2.9
5	do.[2]	do	56	5.6

[1] In front of electric fan.
[2] On rotating table in front of air-pressure exhaust.

TABLE 25.—*Free virus demonstrated in guttation water from tomato and droplets from cut veins and petioles of tobacco induced by root pressure, as well as in precipitation water on leaf surfaces*

[Droplets picked off with glass spatula without touching plant, then wiped on small area of hybrid host]

Origin of droplets [1]	Experiment no.	Droplets tested	Droplets yielding infection	Total lesions	Average number of lesions to droplet
		Number	*Number*	*Number*	
Tomato guttation (artificial)	1	30	26	202	7.7
	2	53	49	365	7.4
	3	17	17	699	41.1
	4	18	14	385	27.5
Tomato cut leaf petiole (artificial)	5	10	10	354	35.4
	6	10	10	246	24.6
Tobacco cut leaf veins (artificial)	7	10	10	558	55.8
	8	10	7	224	32.0
Tobacco surface precipitation water	9	26	14	46	3.3
	10	30	5	13	2.6
Tomato guttation (natural)	11	16	9	74	8.2
	12	10	3	25	8.3

[1] Droplets varied in size from full drop (1/50 cc) to 1/4 of a drop.

The logic of infection and dissemination through wind and rain have been repeatedly tested experimentally in the present connection with corroboratory results. By using the hybrid, results may be secured more rapidly and with more certainty as to the origin of

infection than with ordinary tobacco (table 26). Most of the data presented were secured with young plants set some distance apart in flats and exposed for 1 hour to a regulated air blast on a slowly rotating table, with and without a fine spray of water from above. In this manner, gusts of wind and moisture from all sides were made to whip the plants about on the soil surface to any desired degree without exposure to a constant pressure from one direction. The amount of infection in such trials depends almost entirely upon the amount of virus on or near the soil surface (table 26). Cultivating the soil just prior to the artificial storm favored infection.

On the basis of what is known about the survival and behavior of the virus in the soil (*5*), the situation on infested tobacco land may be readily understood. If the virus in the soil surface has been inactivated by drying, little direct infection through storms may result. On the other hand, in rainy seasons or in stormy periods, and soon after cultivations which bring moist infested soil to the surface, higher infection is likely. In consequence, variations may be expected in infection from season to season or year to year on the same land on the basis of weather conditions during the growing season as well as on the basis of the amount of overwintering virus. General observations on tobacco diseases during drought years have indicated that mosaic is quite as common and abundant as in wet or stormy seasons, and this has led to the assumption that weather has no relation to the amount of mosaic. It is now believed that actual mosaic records would fail to entirely support this conclusion. When the Wisconsin records of mosaic in infested station fields for the last 10 years are compared with the weather records of these years, some correlation between the weather and percentage of mosaic are apparent (table 27). On the other hand, high correlation should not be expected since the environment on the soil surface for the period prior to the storm was not taken into account, and this factor, as has been pointed out, may be expected to influence the amount of direct infection from the soil. This situation was especially striking in the dry season of 1936 (tables 1 and 27), when the percentage of mosaic in infested fields was the lowest ever observed. Further records on the relation of storms to infection and spread of tobacco mosaic are necessary before the actual significance of this factor can be reliably estimated.

TABLE 26.—*Transmission of tobacco virus 1 from infested soil to young plants in flats as a consequence of artificial wind and rain injury*

Flats with 25 young healthy hybrid plants on a slowly rotating table for 1 hour below a fine water spray and a strong air movement from air-pressure line]

Treatment	Virus concentration in soil, lesions on 3 hybrid leaves from sample	Plants infected out of 25 exposed	Total lesions on exposed hybrids	Treatment	Virus concentration in soil, lesions on 3 hybrid leaves from sample	Plants infected out of 25 exposed	Total lesions on exposed hybrids
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
Wind and rain 1 hour	50+	23	135	Wind and rain 1 hour	7	15	30
	23	10	14		7	17	29
	18	7	12		[1] 0	0	0
	15	11	27		[1] [2] 0	21	94
	14	10	15	None	[1] [2] 0	2	2
	13	4	4		[1] 15	0	0

[1] Control. [2] Plants sprayed with virus extract of concentration 350 lesions to 3 hybrid leaves.

TABLE 27.—*Comparative data of heaviest rain and wind storms over a 10-year period with percent of mosaic in subsequent periods in 2 tobacco fields,*[1] *Madison, Wis.*

Year	June				July				August			
	Weather		Mosaic		Weather		Mosaic		Weather		Mosaic	
	Rain, greatest in 24 hours	Wind, maximum velocity	Field 2	Field 7	Rain, greatest in 24 hours	Wind, maximum velocity	Field 2	Field 7	Rain, greatest in 24 hours	Wind, maximum velocity	Field 2	Field 7
	Inches	*Miles per hour*	*Per cent*	*Per cent*	*Inches*	*Miles per hour*	*Per cent*	*Per cent*	*Inches*	*Miles per hour*	*Per cent*	*Per cent*
1926	1.10	37	4.5	-------	0.62	25	8.1	-------	0.44	24	13.8	-------
1927	1.56	31	2.4	-------	.90	28	16.8	-------	.23	24	46.1	-------
1928	2.14	25	8.3	-------	1.78	19	32.1	-------	1.48	36	-------	-------
1929	1.97	23	3.5	1.3	3.90	26	21.2	10.3	.29	22	-------	-------
1930	1.41	24	6.9	3.0	1.58	30	-------	-------	.79	15	56.4	74.0
1931	1.58	40	4.3	2.1	1.26	22	25.0	24.1	3.13	31	83.0	58.2
1932	1.04	21	5.2	1.7	1.75	25	17.0	8.4	1.38	25	66.0	37.4
1933	1.44	25	-------	.6	1.97	31	-------	3.1	1.79	32	-------	17.6
1934	1.02	30	-------	4.1	1.53	21	-------	8.7	1.08	21	-------	19.2
1935	1.51	26	-------	5.5	.84	19	-------	12.2	3.21	21	-------	27.7
1936 [2]	1.07	22	-------	.1	.79	21	-------	3.5	1.98	30	-------	4.5
66-year period	3.47	41	-------	-------	4.32	50	-------	-------	5.07	43	-------	-------

[1] Field 2, data based on about 2,000 to 6,000 plants a year; field 7, about 10,000 plants a year. Weather Bureau records used, field 2 and field 7 being about 1 and 2 miles, respectively, from the Weather Bureau station.

[2] Season of unusual desiccation of surface soil.

DISCUSSION

The local-lesion method of inoculation has permitted a more satisfactory analysis of the factors relating to the control of tobacco mosaic than has hitherto been possible. Field experiments and field surveys, unless conducted with the utmost attention to detail, may easily be misleading unless all the factors which may influence the results are taken into consideration. If, for example, field experiments on the relation of cultivation to infection from the soil are being conducted, the results might be greatly influenced by the seasonal conditions and the time and the depth of cultivation, since if the surface soil is thoroughly dry, the virus originally present may be entirely inactivated. On the other hand, a deep cultivation, bringing up moist, infested soil from below, followed by a storm before this soil has had time to dry out, may result in a comparatively heavy infection. Similarly, transplanting in dry as compared to moist, recently prepared soils may influence the percentage of transmission by hand from the soil to the plant.

If the entire practical problem of mosaic control is examined from all points of view, the obscure nature of its behavior is largely eliminated. The problem has been to explain the escapes from infection quite as much as to explain the infection. The results do not offer any new or simplified methods of control beyond those already known or suspected, but the data have served to either corroborate the more important facts or to reduce the significance of the more doubtfully established conclusions. Manifestly, it is not good practice to attempt to utilize control measures without consideration of their relative importance.

Although differences in cultural methods and practices, workmen, soils, and climate play some part in mosaic control, the evidence indicating the important relation of infested soil to heavy field infection has been corroborated. The early (before topping) infection from this source, amounting as it often does to from 10 to 25 percent of the plants, is more than sufficient to permit further widespread dissemination through ordinary cultural practices (especially topping and suckering). Where it is desired to control mosaic, the avoidance of infested soil which yields high percentages of mosaic will be necessary until mosaic-resistant commercial strains are developed. It is admittedly not always easy to make a decision as to when the soil has become infested to the extent that it is not advisable to continue the land in tobacco. The changing of the tobacco land, considering suitability, influence of preceding crops, fertility, etc., may be quite as undesirable as a moderate percentage of mosaic infection.

The original mosaic infections which develop in the field from other potential sources than that of the field soil appear to be largely avoidable. Although the writer's experiments and observations do not indicate that the use of tobacco by growers plays an important part in field occurrence of mosaic in Wisconsin, it may do so in other States, particularly where leaf tobacco (as contrasted with manufactured tobacco) is handled or used while the new crop is being worked. As has been pointed out, however, this source may be easily eliminated by washing the hands or sterilizing the tobacco used (*13*). The application of stalks, stems, or other refuse likely to contain the virus, to tobacco land less than 5 or 6 months prior to planting is not good practice from the standpoint of mosaic control, although it obviously may not do much additional harm on land already infested.

The production of mosaic-free plants in the seedbed should not be difficult. The use of the ordinary sanitary measures which are recommended and are advisable for the prevention of other tobacco diseases will suffice and are inexpensive. Occasional infections from some unrecognized source apparently creep into the seedbed and may be directly or indirectly responsible for a low percentage of early infection in the field, amounting usually to less than 0.2 percent. When such early infections occur on previously uninfested soil, to the amount of less than 1 percent, early roguing may be quite practical, from the standpoint of reducing further spread in the current crop and increased soil infestation for succeeding crops. Early roguing on soils known to be previously infested with the virus may be of little or no value in reducing the ultimate infection, and, if the roguing amounts to as much as 5 percent, its value may be questioned on the basis of reduction of crop yield.

Considering the relation of the virus concentration in the soil to infection, it seems most likely that the efficiency of fall disking, plowing, or root removal may depend upon the percentage of infection present at the time these operations are performed. If this percentage is low, its greatest value may be in the prevention of further development of the virus in the second growth, which in turn infects the old roots. The additional actual destruction of virus already present, by any of these treatments, probably would never exceed 50 percent of that already present, and generally would be much less.

The dissemination of the virus from soil to plant or from plant to plant in the field through cultural operations is often difficult to avoid. It is not always easy for an expert on mosaic to recognize diseased plants in the field in the early stages of the disease, and it must be remembered that the workmen in the field are often not sufficiently interested in the outcome of the crop to give the constant attention required to detailed instructions of this nature. A certain amount of dissemination is unavoidable in the operations of cultivating, worming, or topping as a consequence of alternate contact with diseased and healthy plants, since the leaves, especially in cigar and burley types, soon reach a stage where the distance between adjoining plants is too narrow to permit these operations without constant contact with the plants. Topping diseased and healthy plants separately is of considerable value, but even with special care under experimental conditions, the percentage of mosaic between topping and suckering stages may be doubled (*9*). Everything considered, the best prevention for such dissemination is the reduction of the original percentage of infection in the field to a minimum. This apparently can only be assured by the production of disease-free plants in the seed bed and transplanting to uninfested or relatively uninfested soil. Just when it is desirable to change the tobacco land to avoid mosaic will vary with circumstances, and this is a problem which the individual grower must decide with the advice he may secure from local authorities on the subject.

When the entire scope of the questions involved in the control of mosaic is realized, the preliminary nature of many of the investigations treated in this paper are recognized. The present need appears to be for more laboratory studies, of the general nature presented here, in other growing districts where mosaic control is a problem, together with a limited number of more detailed field surveys; and, finally, field trials of a demonstrational type. In this manner, it may be possible to reach more universal agreement relative to the comparative and actual value of the various control recommendations offered.

SUMMARY

The factors involved in the survival, infection, and dissemination of the ordinary tobacco mosaic virus (*tobacco virus 1*) have been investigated by the local-lesion method, a hybrid (*Nicotiana tabacum* L.$\times$*N. glutinosa* L.) being used as a test plant.

The experiments are based in part upon an effort to explain the course of development of mosaic under field conditions, as revealed by field records and influenced by cultural practices and weather conditions.

The survival of the virus in infested field refuse and in harvested tobacco has been determined in some detail in relation to the natural and artificial conditions to which such infectious material is exposed. The concentration of virus to which the new crop is exposed is considered to have an important bearing upon the amount of infection resulting.

Some brands of cigars and cigarettes were found to carry fairly high concentrations of virus, but other forms of commercial tobacco carried little or none. Virus in refuse from harvested tobacco and in field refuse may be largely inactivated when fully exposed to weather-

ing and decay for 5 or 6 months. Desiccation in relatively early stages of decay may greatly hasten inactivation.

Under field conditions, the virus was found to survive in high concentrations in the roots until the succeeding crop was planted. The virus in plant tissues above ground when exposed to both decomposition and weathering is inactivated earlier. The virus may survive as long as 2 years in close association with the soil in the absence of freezing and desiccation.

Infection does not take place commonly, if at all, through the roots of tobacco. Infection through the stem and leaves may arise from several different potential sources, the most common of which is the soil. Although direct infection from the soil may rarely exceed 25 percent, subsequent dissemination from plant to plant by various cultural practices accounts for the high percentages of mosaic sometimes observed.

The possible relation of storms to infection and dissemination of mosaic has been demonstrated by laboratory methods.

The practical problems of the control of the ordinary tobacco mosaic are essentially those of locating and reducing the important potential sources of infection. While some benefits may come from attempts at reducing the means of dissemination, after considerable primary infection has become established such spread is often unavoidable under practical field conditions.

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THE ROSETTE DISEASE OF BLACKBERRIES AND DEWBERRIES[1]

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INTRODUCTION

In a previous publication (*5*)[2] a brief, nontechnical account of the rosette disease of blackberries and dewberries with recommendations for its control was given for the use of growers. The purpose of the present paper is to present the experimental data gathered during 5 years of study of the disease and its causal agent.

THE DISEASE

SYNONYMY

The rosette disease is undoubtedly identical with the one described by Cook (*1*, *2*) under the name "double blossom." The double blossom disease has been studied on cultivated blackberries in southern New Jersey and on wild blackberries in Virginia, in the vicinity of Washington, D. C. Isolates of the causal fungus obtained from wild blackberries in Virginia, and from typical double-blossom specimens sent from Florida have been found to be identical with those obtained from "rosette" material in Louisiana and have produced the typical rosette disease when inoculated into healthy plants. The term "rosette", as a name for this disease is preferred for the following reasons: (1) It appears to have priority over "double blossom", since Norton (*4*) used it in an earlier publication; (2) it is descriptive of the symptoms, while double blossom is not, since the affected blossoms are distinctly not double; and (3) the disease has been known by this name for many years in the Louisiana area.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Apparently Earle was the first to recognize the rosette disease. He collected specimens of affected plants (variety not stated) in Cobden, Ill., and sent them to Winter, who in 1885 described the fungus associated with the disease as *Fusisporium* (?) *rubi* (*9*). The disease is not mentioned again in the literature until 24 years later, when in 1909 Norton (*4*) reported it, under the name of rosette, as being serious in Maryland. In 1910 Cook (*1*) reported it, under the name double blossom, as being very destructive to blackberries and dewberries in Delaware, and the following year he (*2*) published a detailed account of it, giving experimental proof of the pathogenicity of the fungus found associated with it. In this account Cook accepted Winter's name for the causal organism but did not attempt to classify it. In the same year, Reimer and Detjen (*6*) reported the disease as

[1] Received for publication July 29, 1936; issued April 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 302.

being serious on dewberries and blackberries in North Carolina. These authors also produced the disease by inoculating healthy blackberries and dewberries with the fungus found associated with it, and a culture of this fungus which was sent to Cook was determined by him to be identical with the one found causing the disease in Delaware. The disease occurs in southern New Jersey where, in some years at least, it becomes very destructive. In addition to the States mentioned above, it is known to occur in Virginia, Kentucky, Ala-

FIGURE 1.—Rosette on wild dewberry (*Rubus trivialis*): *A*, Rosette cane; *B*, healthy canes. Approximately one-third natural size.

bama, Mississippi, Florida, Arkansas, Missouri, Louisiana, and Texas. It is safe to state that the disease is present in all the south and southeast section of the United States, from New Jersey to southeastern Texas, and from the Gulf of Mexico to southern Illinois. How far west it extends is not known. In the herbarium of the Louisiana State University there is a specimen of wild blackberry (species not stated) collected by J. F. Joor in Harrisburg, Tex., in 1876, which shows the typical symptoms of rosette. The collector apparently was not aware that this material was diseased. The

disease was reported from Texas in 1922.[3] Apparently it has never been reported from the Pacific Coast States, where blackberries and dewberries are extensively grown.

In Louisiana the disease is very widespread and very destructive. It occurs in great abundance on wild blackberries and sometimes on wild dewberries. It is especially destructive on cultivated blackberries and dewberries and seems to have been the factor limiting the commercial growing of blackberries and dewberries in that State.

DESCRIPTION

The disease is easily recognized by the rosette or witches'-broom type of growth (fig. 1) which appears on affected canes in the spring. Healthy canes give rise to simple shoots (fig. 1, *B*), while diseased canes produce multiple shoots (fig. 1, *A*) which develop in the form of witches' brooms. The color of the rosette foliage, especially in the later stages, is a lighter green than that of the healthy leaves and often becomes yellowish brown to bronze. In cases of severe infection, every bud of a cane may be so diseased that the entire cane becomes a mass of witches' brooms; more often, however, one or more lateral branches only are affected.

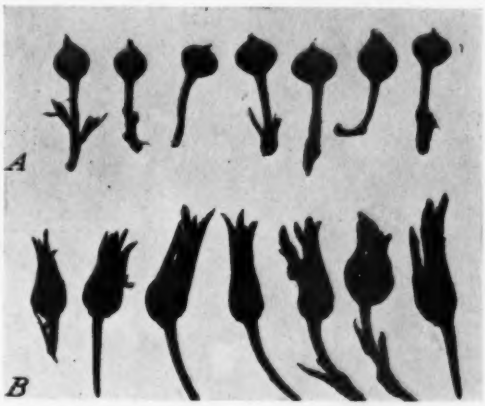

FIGURE 2.—Unopened blossoms from healthy and rosetted plants of *Rubus abundiflorus*: *A*, Healthy; *B*, diseased. Approximately twice natural size.

While as a rule diseased shoots produce a larger number of blossoms, not infrequently the rosette growth may be entirely vegetative. The diseased blossoms are characteristically different from healthy ones. The young, unopened healthy blossoms are round, tightly closed, and compact (fig. 2). The diseased ones are elongated, puffed, larger in size (fig. 2), and almost invariably sterile, although occasionally a few drooplets may set and develop into much deformed berries. The sepals enlarge and occasionally change into leaves (fig. 3, *A*). The petals are also larger and usually more pink than those of the healthy blossoms and are ruffed in such a way as to appear double. Not infrequently the petals fail to unfold. Occasionally, some or all of the petals of a blossom may become green and leaflike. The stamens become abortive early in their growth, seldom reaching the stage when pollen is matured. The pistils are usually larger than those of the healthy blossoms and occasionally the receptacle may fail entirely to develop, leaving a hollow space in the center of the blossom. Sometimes also the receptacle and the pistils may assume a teratological type of growth, the base of the receptacle elongating, stem-

[3] HASKELL, R. J., and WOODS, J. I. BLACKBERRY, LOGANBERRY, AND DEWBERRY. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Sup. 20: 113-114. 1922. See p. 114.

like, and the pistils enlarging and remaining open, leaflike (fig. 3, *B*). Soon after the blossoms open the pistils and stamens become covered with a whitish material which consists of the mycelium and spores of the causal fungus.

The symptoms vary somewhat on different hosts. In the case of the Brainerd blackberry the petals almost invariably fail to unfold (fig. 3, *C*). The Nanticoke blackberry shows the most atypical

FIGURE 3.—*A*, Blossom of Brainerd blackberry affected with rosette, the (sepal have changed into normal leaves); approximately natural size; *B*, blossom of the Gardena dewberry affected with rosette, showing teratological type of growth, base of receptacle elongated, petals green, and carpels opened; approximately one-half natural size; *C*, blossom cluster of the Brainerd blackberry affected with rosette, showing unfolded petals, a characteristic symptom on this variety; approximately one-half natural size.

symptoms. On this variety the shoots may develop singly, like those on healthy plants, or two to three may arise from the same node, but the typical rosette or witches'-broom growth does not form. The affected blossoms, however, as in other hosts, do not set fruits.

HOST SUSCEPTIBILITY

The rosette disease is apparently limited to wild and cultivated species and varieties of the genus *Rubus*. Cook (*2*) found it very severe on the Lucretia dewberry and the Rathbone blackberry.

Reimer and Detjen (*6*) list the Wilson blackberry, the Lucretia dewberry, and a wild blackberry (*R. nigrobaccus*) as affected by the disease in North Carolina. In Louisiana it has been found on the following species and varieties:

BLACKBERRY

Wild species: *R. abundiflorus* and *R. louisianicus* (?).
Cultivated varieties: Brainerd, Crystal White, Joy, Jumbo, Lawton, McDonald, Mersereau, Nanticoke, U. S. D. A. No. 459, and Ward.

DEWBERRY

Wild species: *R. trivialis* and *R. sons*.
Cultivated varieties: Gardena, Lucretia, and Young (Youngberry).

All these species and varieties have been growing side by side in the test plot in Baton Rouge during the last 4 years and it has been possible to make observations on their comparative resistance or susceptibility. All the cultivated varieties of blackberry, the wild dewberry *Rubus sons*, and the cultivated dewberry varieties Gardena and Lucretia, were found to be very susceptible. The wild blackberries, *R. abundiflorus* and *R. louisianicus*, and the Young dewberry are somewhat less susceptible. The wild dewberry *R. trivialis* appears somewhat resistant, and the disease only rarely occurs on it in nature. If inoculated with the causal organism, however, it takes the disease very readily and very severely (fig. 1). Consequently this species seems to escape infection rather than to possess any inherent resistance. It is believed that it escapes infection because of its procumbent habit of growth. Its primocanes (new canes) are usually covered by weeds and are not exposed to the spores of the fungus. Moreover the early primocanes are weaker than those produced later in the season after the period of infection has passed and are usually smothered by the later rank growth. The Himalaya blackberry appears to be immune. No sign of the disease was ever noted on this variety, although it has been growing by the side of severely diseased plants in the test plot for 4 years. The Brainerd, on the other hand, which has the Himalaya as its maternal parent (*3*), has been found very susceptible under Louisiana conditions, although Darrow and Waldo (*3*) state that the disease is not serious on it in North Carolina.

THE CAUSAL FUNGUS

CLASSIFICATION AND NOMENCLATURE

Winter (*9*) first described the organism associated with the rosette disease from material sent to him by Earle from Cobden, Ill., in 1885 as *Fusisporium* (?) *rubi*. That there was doubt in Winter's mind about placing the fungus in the genus *Fusisporium* is evident from the fact that in his description he placed a question mark after the generic name. Winter describes the spores of this fungus as 14μ–30μ×3.0μ–3.5μ in size and one to three septate. Three years later Earle distributed specimens (Seymour and Earle's Economic Fungi, no. 25) of additional material collected at Anna, Ill. (not far from Cobden). According to Cook (*2*) this material was from *Rubus villosus* Ait. var. Wilson's Early blackberry. In 1911 Cook (*2*), in his account of the "double blossom" disease, described the fungus which he found causing this disease. Although the spores of his fungus were much

longer and had more septa (14μ–53μ long, one to eight septate) than those of the one described by Winter, Cook considered the two to be identical. The fact that Winter's and Cook's measurements of the spores do not agree is not considered significant because, as is brought out later in this paper, the fungus is extremely variable.

In 1917 Wollenweber (*10*) reexamined Winter's original specimens, and changed the name of the fungus to *Ramularia rubi* (Wint.).

Although Winter's original specimens have not been seen by the writer, a specimen of Earle's later material was obtained through the courtesy of C. L. Shear, formerly principal pathologist, United States Department of Agriculture, for comparison with the Louisiana fungus. This specimen, which consisted of one blossom, was labeled as follows:

Fusarium rubi Wint.
On *Rubus villosus* (cult.)
Causing "Double Blossom."
Anna, Illinois. June 13, 1888.
F. S. Earle
(*Ramularia rubi* (Wint.) Wollenweber)
See Sherbakoff

The macroscopic appearance of this specimen was typical of blossoms affected with rosette in Louisiana. An examination of 100 spores from this specimen gave the following results:

Spore size: 13.6μ–64.6μ×3.06μ–3.74μ; average 30.36μ×3.30μ.
Septation: 0–7; average 2.7 septa per spore.
Shape: Ratio of straight to curved spores, 1:1.09.

These measurements agree rather closely, considering the extreme variability of this fungus, with the measurements obtained from the Louisiana material. The conidiophores, also, were similar to those from Louisiana material (fig. 4, *C*).

It is evident that Winter's tentative classification of the fungus was incorrect. The fungus plainly is not a *Fusarium* (*Fusisporium*). The question then is, since its perfect stage is not known, whether it should be placed in the genus *Ramularia*, as was done by Wollenweber (*10*), or in the genus *Cercosporella*. Some of the spores, especially of certain microsporic strains, are typical of the genus *Ramularia*—hyaline, oblong, cylindric (fig. 4, *D–F*). On the other hand, the hyaline, long filiform, decidedly curved, tapering spores (fig. 4, *G–L*) characteristic of many strains, are typically those of *Cercosporella*. The sporophores (fig. 4, *A–B*), although short, are of the *Cercosporella* type. For these reasons it is believed that the fungus fits better in the genus *Cercosporella* than in *Ramularia*, and is therefore, designated as *Cercosporella rubi* (Wint.) comb. n.

DESCRIPTION

The mycelium is hyaline, septate, very slender, generally 1.5μ or less in diameter, although some branches of aerial hyphae may be thicker, 2.0μ–2.5μ. The hyphae anastomose freely, forming a rather dense mycelial network. The anastomosing occurs both in culture and within the host. In culture the network is cemented together by a jellylike substance and the colonies are of a rather tough,

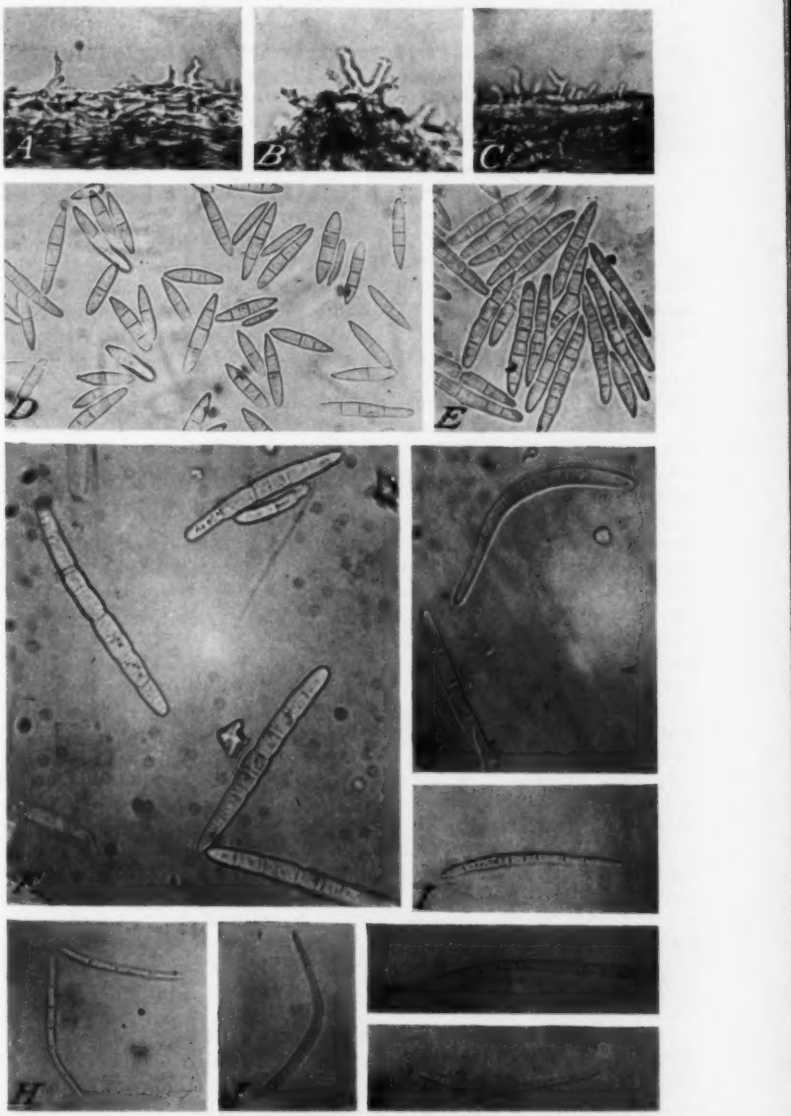

FIGURE 4.—*Cercosporella rubi: A* and *B*, Sporophores from blossoms of the Lucretia dewberry, Hammond, La.; × 460. *C*, Sporophores from Earle's specimen, Anna, Ill., 1888; × 296. *D*, Short type of spores, mostly three-septate, from blossoms of *Rubus abundiflorus*; × 360. *E*, Long, constricted, mostly multiseptate spores, from blossom of Nanticoke blackberry; × 475. *F*, Spores from blossom of the McDonald blackberry similar to those in *E*; × 655. *G*, Long, curved, tapering type of spores from blossom of Young dewberry, Franklin, La.; × 655. *H*, Spores from Young dewberry blossoms from Baton Rouge, La., similar to those in *G*; × 200. *I–L*, Long, curved, tapering type of spores from blossoms of Gardena dewberry, Hammond, La.; × 450.

slippery, rubbery consistency. The fungus grows well on a variety of media (Czapek's, bean-pod, potato-dextrose, and corn-meal agars and autoclaved blackberry stems and bean pods). It makes its best growth on bean pods. The fungus is slow growing, and the colonies have never been very large on any of the media tried. It sporulates fairly well in culture, but never so abundantly as it does in nature on withered blossoms.

The fungus is extremely variable in regard to length, curvature, and degree of septation of the spores. The following examples illustrate this variability:

(1) Spores from one blossom of naturally infected Young dewberry, collected in Baton Rouge, April 19, 1935; 100 spores were studied. Length: 17.0μ–85.0μ, average 40.9μ. Septa: 1 to 7, average 2.8 per spore. Shape: 72.5 percent curved, and most of the spores decidedly tapering (fig. 4, *G*, *H*).

(2) From the same material as in (1), but from a different blossom. Length: 13.6μ–37.4μ, average 21.1μ. Septa: 0 to 4, average 1.9 per spore. Shape: 22.9 percent curved, and most spores blunt.

(3) From the same material as in (1) and (2), but from a different blossom. Length: 20.4μ–81.6μ, average 36.0μ. Septa: 1 to 9, average 3.6 per spore. Shape: 55.0 percent curved, and most spores pointed.

These examples show the necessity of examining more than one sample of material in order to get a true picture of the characters of a variable organism. It is probably that Winter, in his original description of this fungus, happened to examine a specimen that contained the short type of spores.

Similar variations were noted in material from other varieties of blackberries and dewberries (McDonald, Nanticoke, Lawton, Brainerd, Jumbo, Gardena, Lucretia) collected from different localities and on different dates from April 19 to May 11, 1935. Measurements were made of 18,385 spores from 186 blossoms (approximately 100 spores from each blossom). Wide variation in the length, shape, and degree of septation of the spores from different blossoms and even between the spores from two adjacent blossoms on the same flower cluster was observed.

The smallest averages found in any one of the 186 groups for length, septation, and percentage of curved spores were 21.1μ, 1.9 septa per spore, and 18.0 percent curved spores and the highest were 53.2μ, 4.9 and 88.0 percent, respectively. A summary of the measurements for the 186 groups follows:

Length: 17.1μ to 57.6μ, average 33.1μ. Septa: 0 to 11, average, 2.97 per spore. Percentage of curved spores: 18.0 to 88.0, average, 48.36.

Groups with short spores predominated. The minimum length of spores lies within a relatively narrow range, 81.5 percent of the mean minima of 186 separate lots of spores measured (approximately 100 spores per lot) ranged between 13.0μ and 19.0μ. The average length of spores also was relatively narrow, 87.0 percent of the averages of the same 186 lots ranging between 25μ and 40μ. The mean maximum length of spores, however, was very wide, between 30μ and 96μ, and so irregular that when the mean maxima of the 186 lots were plotted a broken zigzag curve was obtained.

That the variations noted in this fungus are not constant is shown by a case noted in April 1934. While making isolations from rosette blossoms of the wild blackberry *Rubus abundiflorus* one isolate was obtained which differed from the others in two important respects. It sporulated very profusely in culture, which is not a characteristic of this fungus; and its spores were much longer than those of the other isolates. The spores averaged 62.7μ in length, approximately twice that of the average length of spores of most isolates. It was subcultured on different media (bean-pod, Czapek's, Cunningham's, and potato-dextrose agars) several times for a period of over 1 year, and it continued to sporulate profusely, producing long spores.

This isolate was then used to inoculate healthy primocanes of the Young dewberry. It produced infection, showing about the same degree of pathogenicity as other isolates. However, when measurements were made of spores produced on the blossoms it was found that they were of approximately the same size, shape, and degree of septation as those produced on plants inoculated with other isolates or those produced by natural infection. Furthermore, none of the reisolations made (52 in all) from blossoms of the plants inoculated with the long-spored strain have produced long spores. Therefore, the fungus has reverted to type after passage through the host.

Other measurements of spores from composite samples (a spore suspension was made from several blossoms from each sample) made at different times during 1933 and 1934 from different varieties of blackberries and dewberries, gave results which agree very closely with those for the 186 groups shown above. These were as follows: Number of spores measured, 900; average size of spores, 34.8$\mu\times$3.7μ; range in size of spores, 13.6μ–69.0$\mu\times$2.7μ–4.4μ; range in septation, 0–12; average number of septa per spore, 3.04; ratio of straight to curved spores, 1:1.04.

The conidiophores (fig. 4, *A*, *B*) are hyaline, rather short, mostly simple, occasionally branching, rarely septate, 3.4μ–4.2$\mu\times$5.0μ–27.0μ, average 3.8μ–12.7μ. Measurements of conidiophores from different samples gave the following figures: Number of conidiophores measured, 130; average size, 3.8$\mu\times$12.7μ; range in size, 3.4μ–4.2$\mu\times$5.0μ–27.0μ; septate (1 septum), 20; branched, 7; spore scars, 1–7, average per conidiophore 2.8.

REVISED TECHNICAL DESCRIPTION

Cercosporella rubi (Winter) comb. n.

Mycelium septate, hyaline, thin, generally 1.5μ or less in diameter, aerial hyphae thicker, 2.0μ–2.5μ in diameter, anastomosing very freely both in culture and within host buds, forming netlike type of growth. In culture colonies usually slimy and of tough, rubbery texture. Conidiophores arise as side branches, singly or in fascicles, hyaline, short, usually simple, rarely branched, rarely septate (1 septum), 3.4μ–4.2$\mu\times$5.0μ–27.0μ, average 3.8$\mu\times$12.7μ, and with 1 to 7 spore scars. Conidia hyaline, cylindric to tapering, straight to curved, 0–12 septate, averaging about 3 septa per spore, with or without constrictions at septum, very variable in length, 13μ–96$\mu\times$2.7μ–4.7μ, average 33.8$\mu\times$3.8μ. Mycelial stage occurs within vegetative and floral buds of host, sporulating profusely on opened, withering blossoms. Sporulation in culture usually sparse. On wild and cultivated species and varieties of *Rubus*, causing "rosette" or "double blossom" disease.

EFFECT OF TEMPERATURE ON THE FUNGUS

The effect of temperature on the rate and type of growth and on the sporulation of the fungus in culture was determined. Cultures

were incubated at the following temperatures: 6°, 9°, 15°, 17°, 21° 27.5°, and 31° C. For the most part, large agar slants, 50 cc of agar in 125-cc flasks, were used in order to prevent rapid drying of the medium, especially at the higher temperature ranges, since the fungus is a slow grower and several days must elapse before it makes measurable growth. However, agar plates and tube slants were also used. Bean-pod agar was found to be a favorable medium for the fungus and was used in the temperature studies.

The results obtained, based on the averages of several trials, are shown graphically in figure 5. Readings were taken at the end of 1 and 2 weeks. The optimum temperature for growth was about 25° C. The fungus grew fairly well at 27.5° to 28°, but growth practically stopped at 31°. No measurable growth at the end of 7 days, and only traces of growth at the end of 14 days, was observed at this temperature. The minimum temperature at which there was growth appears to be between 6° and 9°. Only traces of growth had occurred at 6° by the end of 14 days and no apparent growth by the end of 7 days.

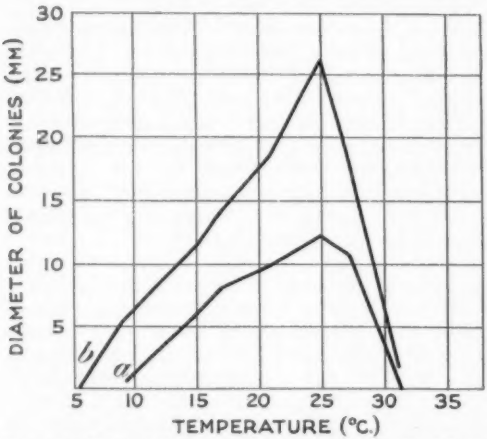

FIGURE 5.—Effect of different temperatures on the growth of *Cercosporella rubi* in culture. Diameter of colonies (*a*) at the end of 1 week; (*b*) 2 weeks.

The influence of temperature on the growth of the fungus is considered important, for it is believed that high temperature is one of the factors limiting the period during which infection takes place. This point is discussed in more detail later in this paper.

No spores were produced at temperatures of 6°, 9°, and 15° C. Sporulation was at its maximum between 21° and 25°, and again became less at 27.5°. There seemed to be some difference in the degree of sporulation in the different containers. At the lower marginal temperature range (15° to 17°) spores were fairly plentiful in the Petri dish cultures, but very few were found in the tube and none in the flask cultures.

Temperature and, apparently, the moisture content of the medium had a pronounced effect on the type of growth of the fungus. At the lower temperatures (15° to 17° C.) where the moisture content of the medium in the three types of containers (tubes, plates, and flasks) was approximately equal owing to the low rate of evaporation, the colonies in all three types of containers were about the same in appearance—raised with white, somewhat cottony aerial growth. At 25° the colonies on the plates were flat, spreading, with white, dry, somewhat powdery surfaces. In the flasks the colonies were much

raised, their centers contained hornlike outgrowths twisting counterclockwise, and their margins were slimy. The colonies in the tubes were intermediate in appearance between those on the plates and those in the flasks. At the still higher temperature (27° to 28°) the colonies on the plates were thin, spreading, white to grayish, felty to powdery. In the flasks all the colonies, except small areas in their centers, were slimy.

CYCLE OF THE DISEASE

Infection takes place on the primocanes in the spring from spores produced on the blossoms. The spores fall on the young buds, germinate, and the mycelium penetrates and grows within the buds, filling the spaces between the embryonic bud elements. As the infected buds grow and give rise to secondary or lateral buds, the mycelium also grows and follows and invades the new buds as they are formed. Later in the season, when flower buds begin to differentiate, these also are invaded. The infected primocanes grow normally the first season, showing no external symptoms during the summer and fall,[4] but on resuming growth the following spring the infected buds form the rosette or witches'-broom type of growth. When the infected blossoms open, spores in great profusion are produced on them, chiefly on the stamens and pistils, and the cycle is completed. Thus, the fungus exists in the mycelial stage within the buds for about a year and sporulates on the blossoms only.

PATHOGENICITY

The pathogenicity of this fungus was first established by Cook (*1*, *2*) and later by Reimer and Detjen (*6*). The results obtained in Louisiana fully confirm those of these previous workers. Infection experiments were carried on from 1931 to 1935, the results of which are here presented.

THE 1931 INOCULATION TESTS

Thirty-six plants of wild blackberry (*Rubus abundiflorus*) which were kept under observation for 2 years and were known to be free of rosette were treated as follows: (1) 12 were inoculated on May 18 by spraying the primocanes with a water suspension of spores and mycelium from a 10-day-old culture of the fungus on potato-dextrose agar. (2) These 12 plants and 8 additional ones were inoculated in the same manner on July 15, August 17, October 3, November 16 and 20, January 15, and February 1. The plants were growing outdoors. (3) Sixteen untreated plants were kept as checks.

The results of the inoculations were recorded on February 19, 1932. The surviving 13 of the original 16 noninoculated check plants and the 8 plants that were included in the inoculation scheme beginning July 15 remained entirely free of rosette.

Of the 12 plants which were inoculated first on May 18 and also on the subsequent dates indicated, 10 survived and 2 died. Of the surviving 10, 7 were affected with rosette in varying degrees of severity, from 2 buds on one plant to all the buds on some of the other canes. Three of the 10 remained free of rosette.

[4] In some years, when the weather is mild in late fall, the infected buds may begin to grow prematurely and show the rosette symptoms. Otherwise the symptoms do not appear until late February or early March.

The results of this more or less preliminary test indicated that the fungus was capable of producing the disease, and that infection apparently took place early in the season, since only the plants that were included in the first inoculation (May 18) contracted the disease, all others remaining healthy even though inoculated seven different times.

TABLE 1.—*Plan and results of the 1932 inoculation experiments*

Date inoculated	Number and kind of plants inoculated	Inoculum [1]	Results [2]
Apr. 22	10 wild blackberries (*Rubus abundiflorus*)	A	Severe infection, 8; no infection, 2.
	13 wild dewberries (*R. trivialis*)	A	Severe infection, 7; no infection, 2; dead, 4.
	26 canes of *R. abundiflorus*	B	Severe infection, 8; light infection, 2; no infection, 8; dead, 8.
May 9	7 plants of *R. abundiflorus*	C	Very severe infection (practically every bud on every cane diseased), 1; no infection, 6.
	10 plants of *R. abundiflorus*	A	Infected, 5; healthy, 3; dead, 2.
	8 plants of *R. abundiflorus*	B	Infected, 6; healthy, 2.
	12 plants of *R. trivialis*	B	Infected, 10; dead, 2.
	9 canes of *R. abundiflorus*	C	Infected, 5; healthy, 4.
May 19	23 canes of *R. abundiflorus*	A	Infected, 18; healthy, 5.
	10 canes of *R. abundiflorus*	B	Infected, 10.
June 3	15 plants (90 canes) of *R. abundiflorus*	B	Severe infection, 1; light infection, 3; no infection, 11.
Sept. 3	5 plants of *R. abundiflorus*	B	Very slight infection, only 2 buds on one plant.
	2 dewberry plants (Young variety)	B	Very slight infection, 1 rosette bud on each plant.
Sept. 18	2 plants of *R. abundiflorus*	B	No infection.
	1 dewberry plant (Young variety)	B	Very slight infection, only 2 buds.

[1] A = Water suspension of spores from naturally infected blossoms; B = water suspension of spores and mycelium of *Cercosporella rubi* from pure cultures; C = dry inoculum of spores from naturally infected blossoms.

[2] The term "dead" refers to individual canes and not to the entire plant. Dying of canes was not due to rosette. The early spring canes are often smothered by the more vigorous later growth.

THE 1932 INOCULATION TESTS

The scheme of the 1932 inoculation experiments and the results obtained are shown in table 1. Equal numbers of noninoculated control plants (not shown in the table) were provided in every case. All the control plants remained healthy. The plants used in this experiment were located outdoors. They were all young plants, set out in January 1932, and were known to be free of rosette. In these, as well as in subsequent infection experiments, the following methods were used for inoculating the plants:

(1) The dry-inoculum method: Dry infected blossoms were crumbled in a dish, and the debris (which consisted of spores and mycelium of the fungus, and pieces of the flower tissues) was picked with a brush and placed on the buds of the canes to be inoculated, at the same time pressing gently with the brush so as to tease the bud scales open to allow the inoculum to lodge between them.

(2) The wet-inoculum method: The wet inoculum consisted of a water suspension of spores and mycelium of the fungus, made either from pure culture or from naturally infected blossoms. The inoculum was either sprayed on the canes by means of a small insect sprayer, or placed in a flat pan and the canes dipped in it. In either case, the buds were teased with a brush so as to allow the inoculum to lodge between the scales.

As shown in table 1, infection was secured repeatedly, both with pure cultures of the fungus and with spores from naturally infected blossoms. It should be noted that only slight infection resulted

from the September inoculation. This fact, which was also brought out by other experiments, is important because it has a bearing on the length of the period during which infection may take place.

THE 1933 INOCULATION TESTS

In the 1933 infection experiments, only pure-culture inoculum was used. The kind and number of plants inoculated and the results obtained are summarized in table 2. The plants were located outdoors. Corresponding noninoculated control plants (not included in the table) were provided in every case. The control plants remained healthy except the Crystal White variety in which one small lateral on one plant and two buds on another were rosetted, and the Mersereau variety in which three buds on one plant were rosetted, apparently the result of natural infection.

As shown in table 2, infection was secured very readily on all species and varieties inoculated from May 3 to June 22. No infection resulted from the inoculations made during July and August, and only slight infection from the September 28 inoculation. Rather heavy infection was obtained from the inoculation made on October 17.

TABLE 2.—*Plan and results of the 1933 inoculation experiments; pure inoculum was used*

Date of inoculation	Number and kind of plants inoculated	Results
May 3	36 canes of wild blackberry (*Rubus abundiflorus*).	Infected canes, 21; healthy canes, 14; dead canes, 1.
May 8	54 canes of wild dewberry (*R. trivialis*).	Infected canes, 19; dead canes, 35.
May 23	15 canes of Crystal White blackberry	Very severe infection; practically every bud on every cane diseased.
June 5	32 canes of *R. abundiflorus*	Infected canes, 16; healthy canes, 13; dead canes, 3.
Do	18 canes of blackberry, U. S. D. A. Selection No. 459.	Very severe infection; practically every bud on every cane diseased.
Do	9 canes of Mersereau blackberry	Do.
June 7	1 plant (6 canes) of *R. sons*	Very severe infection, every cane infected.
June 22	28 canes of *R. abundiflorus*	Infected canes, 19; healthy canes, 6; dead canes, 3.
July 6	16 canes of *R. abundiflorus*	No infection.
Do	20 canes of Young dewberry	Do.
July 24	16 canes of *R. abundiflorus*	Do.
Aug. 15	22 canes of *R. abundiflorus*	Do.
Aug. 29	19 canes of *R. abundiflorus*	Do.
Sept. 28	17 canes of *R. abundiflorus*	2 buds on 1 cane and 3 on another infected; all others healthy.
Oct. 17	14 canes of R. *abundiflorus*	Infected canes, 8; healthy canes, 4; dead canes, 2.

THE 1934 INOCULATION TESTS

The plants employed in the 1934 infection experiments were located outdoors, in the same place as those used in the infection tests of the previous year. The results are given in table 3. Adequate control plants were again provided, but, for the sake of brevity, these are not included in the table. All the control plants remained healthy except the Lucretia dewberry on which an occasional rosette bud occurred, the result of natural infection.

The results obtained are comparable to those of the previous year. Infection was secured readily from May 19 to June 10. Practically no infection occurred from the inoculations made from June 30 to September 25. Since an occasional rosette bud appeared on the noninoculated control plants of the Lucretia variety, it is very probable

that the slight amount of infection noted on some of the plants of this variety inoculated on June 30 and August 13 (table 3) resulted from natural infection earlier in the season. This supposition is strengthened by the fact that in none of the previous tests was infection secured from inoculations made during August.

SUMMARY OF THE RESULTS OF ALL INFECTION TESTS

The results obtained from 4 years' inoculation experiments (1) establish the relation of *Cercosporella rubi* as the causal agent of the rosette disease of blackberries and dewberries, and (2) demonstrate that there is a period, from the latter part of June to the latter part of September during which infection either does not take place at all, or takes place with difficulty. The latter fact is very important from the standpoint of control, especially since other experiments have shown that the period during which natural infection occurs is even shorter than that obtained in the inoculation tests.

TABLE 3.—*Plan and results of the 1934 inoculation experiments*

Date inoculated	Number and kind of plants inoculated	Inoculum [1]	Results
May 19	6 Young dewberry plants (19 canes)	A	Severely infected, 4 plants; healthy, 2 plants.
May 30	10 Young dewberry plants (46 canes)	A	Every inoculated cane became infected.
June 5	5 Young plants (27 canes)	B	Do.
Do.	8 Young plants (36 canes)	C	Infected, 7 plants; healthy, 1 plant.
Do.	5 Young plants (30 canes)	A	Infected, 3 plants; healthy, 2 plants.
June 10	6 Young plants (28 canes)	A	Infected, 8 canes; healthy, 20 canes.
Do.	9 Lucretia dewberry plants (42 canes)	A	Infected, 16 canes; healthy, 2 canes; dead, 24 canes.
June 30	9 Lucretia plants (54 canes)	A	32 of the 54 inoculated canes died; the surviving 22 were healthy except for 1 rosette bud on each of 2 canes.
July 21	10 plants of *Rubus abundiflorus*	B	No infection.
Do.	10 Young plants (56 canes)	B	Do.
Aug. 13	10 Lucretia plants (47 canes)	C	23 of the 47 inoculated canes died; the surviving 24 canes were healthy except for 2 rosette buds on 1 cane and 3 on another.
Do.	6 Lucretia plants (32 canes)	B	13 of the inoculated canes died; the surviving 19 canes were healthy except for 3 rosette buds on 1 cane.
Sept. 6	10 plants of *R. abundiflorus*	B	No infection.
Sept. 26	10 Young plants (62 canes)	B	Do.

[1] A = Water suspension of spores from naturally infected blossoms, not a pure culture; B = water suspension of mycelium and spores of a long-spored isolate (B-307) of *Cercosporella rubi* from pure cultures; C = water suspension of mycelium and spores from pure culture of a normal-spored isolate of *C. rubi*.

SPRAYING EXPERIMENTS

THE 1931 EXPERIMENTS

In 1931, before the pathogenicity of *Cercosporella rubi* had been established, two spraying experiments were carried out which demonstrated that bordeaux mixture would control the disease effectively if the spraying was done at the proper time.

The first experiment was conducted on a small planting of blackberries and dewberries at the Fruit and Truck Experiment Station in Hammond, La. The plants had been set out about 5 years before the experiment began, and in the spring of 1930 and 1931 were so badly infected that practically every fruit cane on every plant was severely rosetted. This planting offered an exceptionally favorable testing ground for determining the efficacy of spray.

The planting consisted of 15 short rows (approximately 14 plants to the row) made up of 8 rows of McDonald blackberries and 7 rows of Lucretia dewberries. Eight rows (four of blackberries and four of dewberries) were sprayed and seven alternate rows (four of blackberries and three of dewberries) were left unsprayed as checks.

The spraying with 4–4–50 bordeaux, which was confined for the most part to the primocanes, although some of the spray reached the fruit canes, was done on the following dates: In 1931 on May 6 and 13, June 10, 18, and 29, July 21, August 4, 13, and 28, September 8 and 22, October 18, November 9 and 23, December 11, and in 1932 on January 8, February 3, 10, and 18.

The reason for this extensive spraying, which as a control measure was admittedly economically impracticable, was to determine whether the disease could be controlled. At the time of the spraying the cause of the rosette disease was not known and it was reasoned that if a fungus were the causal agent, it should be possible to prevent infection if the canes were kept covered with spray throughout the year.

The results were recorded in March 1932, although the nonsprayed plants began to show rosette symptoms as early as the last part of December 1931. Very sharp and clear-cut results were obtained. Both blackberries and dewberries of the nonsprayed checks were very severely rosetted. The sprayed blackberries were entirely free of rosette, and all except one of the sprayed dewberriers were free of rosette. On this one plant practically every bud on the three canes which developed was rosetted. While this one exception was very puzzling and could not be explained at the time, subsequent work has shown that, under certain conditions, the fungus may become systemic in the crown of dewberry plants and when this occurs spraying has no effect.

The second spraying experiment was conducted in a commercial planting near Baton Rouge. This planting, consisting of McDonald blackberries and Lucretia and Young dewberries, was so severely affected with rosette in the spring of 1931 that the yield was reduced by about 60 percent.

Portions of 8 rows of dewberries (63 Young and 70 Lucretia plants) and portions of 4 rows of blackberries (McDonald) were sprayed with 4–4–50 bordeaux on the following dates: October 22, November 5, December 5 and 22, 1931, and January 16 and 28 and February 6, 1932. No control whatsoever was obtained. The sprayed plants (both the blackberries and the dewberries) showed about as much rosette (general and severe infection) as the nonsprayed checks.

The results of these two rather preliminary tests indicated that bordeaux mixture is effective in preventing infection if the spraying is done in the spring. Late spraying was absolutely of no value. Subsequent studies have shown that spraying to be effective must be done before June.

THE 1932 EXPERIMENTS

In 1932 spraying was limited to a short period, May 12 to June 1. Thirty-one plants of the McDonald blackberry were sprayed with 4–4–50 bordeaux mixture three times, May 12 and 23 and June 1. Thirty-six other plants of the same variety in adjacent rows were left unsprayed as checks.

All the sprayed plants remained healthy except one, which had one rosette bud. Rosette was very severe on practically every cane of

the nonsprayed checks. On some laterals every bud was diseased. While the number of plants used in this experiment was rather small, the results obtained were so clear-cut as to leave no doubt about the effectiveness of spring spraying with bordeaux mixture for controlling this disease. The experimental plants were located in the same planting as those of the first 1931 spraying test (in Hammond), where the rosette was very severe and where there was an abundance of inoculum for natural infection. The results also brought out the fact that the period during which natural infection takes place is limited. Although no spraying was done after June 1, no infection occurred on the plants that had been sprayed up to that date.

THE 1933 EXPERIMENTS

Table 4 shows the plan and the results of the 1933 spraying tests. "Instant" bordeaux (7) spray was used. The experiment was located in Hammond on a planting where rosette was very prevalent.

TABLE 4.—*Plan and results of the 1933 spraying experiments*

Dates of spraying	Number and kind of plants sprayed	Results
May 6, 15, 25, and June 1	18 McDonald blackberry	All healthy.
Do	25 Lucretia dewberry	All healthy except for 2 rosette buds on 1 plant and 1 on another.
May 25, June 1 and 15	43 McDonald blackberry	Healthy, 31 plants; 1 rosette bud per plant, 7 plants; 2 to 8 rosette buds per plant, 5 plants.
June 1 and 15	45 McDonald blackberry	Every plant showed some rosette, but less than the nonsprayed controls.
Nonsprayed controls	86 McDonald blackberry	Every plant rosetted, some very severely.

The results of these tests corroborate those of the previous 2 years. They show that if spraying is started early in May practically 100-percent control of the disease may be obtained. It was rather surprising that good control was obtained by the relatively late sprayings (May 25–June 15), and that even as late a spraying as June 1–15 gave a certain, although limited, degree of control. This would indicate that considerable time may elapse between the lodging of the spores on the bud scales and invasion of the interior of the bud by the mycelium of the fungus, and that during this period the spray may reach and kill some of the germinating spores. Weather conditions, chiefly moisture, may influence the length of this period.

Comparable results to those of the previous 3 years were obtained in the spraying tests of 1934 and 1935. However, since these latter tests were located on a young planting (in Baton Rouge) where the rosette was not very prevalent, the results were not very sharp and clear-cut; the nonsprayed control plants often either remained healthy or showed only a small amount of infection. In general, the results of these tests indicate that practically absolute control is obtained by spraying about three times at approximately 10-day intervals from the first week in May to about June 1. In one case in the 1934 spray tests, a small amount of infection occurred on young plants sprayed May 16, 26, and June 5, which indicated that, in this particular year at least, May 16 was probably too late to start the spraying.

PRUNING EXPERIMENTS

That the period during which infection may take place is limited was brought out by both the inoculation and the spraying experiments. This period was also determined by pruning tests. Since infection takes place on the new growth (primocanes), removal of the primocanes at different periods offers an exact method for determining the time of infection. If, for example, the primocanes of one set of plants are removed on May 1, and those of a second set of plants on May 20, and in the following spring it is found that the plants of the first set are diseased while those of the second set are not, it is reasonable to assume that infection must have occurred sometime between May 1 and 20.

A large number of pruning tests were made during the 3-year period 1932–34. The following species and varieties were included: Wild blackberry (*Rubus abundiflorus*); cultivated blackberry, McDonald, Brainerd, Joy, Nanticoke, and U. S. D. A. No. 459; wild dewberry, *R. trivialis* and *R. sons*; cultivated dewberry, Lucretia and Young. While space does not permit a detailed account of all the pruning experiments, the following examples should suffice to illustrate the limited time during which infection takes place.

1932 EXPERIMENTS

In 1932, in a small planting of badly rosetted McDonald blackberries where there was an abundance of inoculum present, the primocanes of 15 plants were pruned to the ground on May 3, those of a second set of 13 plants on May 23, and those of a third set of 18 plants on July 1. Control plants (not pruned) were also provided.

The results of this test were recorded in March 1933. The plants pruned on May 3 showed about as much rosette as the nonpruned controls. Those pruned on May 23 were almost free of rosette. Of the 13 plants of this set, 9 showed no rosette at all and 4 showed only a trace (1 to 3 rosette buds per plant). The plants pruned on July 1 were entirely free of rosette. The control (not pruned) plants were very severely rosetted.

1933 EXPERIMENTS

In 1933, 16 Lucretia and 47 McDonald plants were pruned to the ground on May 25. This test was located in the same planting as the previous one, where the disease was very prevalent. Ninety-two McDonald plants were left unpruned as controls.

Practically complete control was obtained by pruning on May 25. All of the 16 Lucretia and 44 of the 47 McDonald plants were entirely free of rosette. The other McDonald plants showed one rosette bud each. The control (nonpruned) plants were very severely rosetted, some rosette buds showing on every cane.

In 1929 two rows of young dewberry plants were planted at the Fruit and Truck Experiment Station at Hammond. Rosette appeared on this planting the following year, and the infection increased considerably during the succeeding years. In 1933, when a pruning experiment was undertaken, the rosette was so prevalent that hardly a healthy cane could be found. These plants, therefore, were excellent experimental material for testing the effectiveness of pruning.

All the fruit canes (last year's growth) were removed and burned on May 15. This eliminated the source of infection from the im-

mediate vicinity. Then the plants were divided into three sets as follows: (1) Twenty-six plants, primocanes pruned May 17; (2) 28 plants, primocanes pruned May 25; (3) 36 plants, control, not pruned. Half of the plants in each set were sprayed with bordeaux mixture on June 1 and 15.

All the pruned plants were entirely free of rosette except one plant in the set pruned May 17. This plant had eight canes, of which two were completely healthy and six on the opposite side of the crown were so severely rosetted that every bud was diseased. This phenomenon of one plant being severely diseased while all the others were entirely healthy was puzzling at the time, for it was evident that it was not the result of later infection. If infection had taken place on the new primocanes which developed after pruning, random distribution of the disease would be expected. Later studies have shown that in the case of the dewberry, infection may become systemic in the crown or in part of the crown, and it is apparent that this one plant was systematically infected.

The nonpruned control plants, both sprayed (June 1 and 15) and unsprayed, were heavily rosetted. There was no noticeable difference in the amount of disease on the sprayed and nonsprayed control plants.

In 1933 a large number of pruning tests were made from May 9 to June 5 with the wild blackberry *Rubus abundiflorus*, the wild dewberry *R. trivialis*, and the Young dewberry. The results of these tests were not very conclusive because the experiments were located on a new planting where the rosette was not very prevalent, and there was very little infection even on the control plants. However, in no case did rosette occur on any of the plants pruned during this period, while some infection, though light, occurred on the controls.

1934 EXPERIMENTS

In 1934 the pruning tests covered a longer period, from May 12 to August 13. The following plants were included: Wild blackberry *R. abundiflorus*, wild dewberry *R. trivialis* and *R. sons*, Young dewberry, and the McDonald, Brainerd, Joy, Nanticoke, and U. S. D. A. No. 459 blackberries. Results similar to those of the previous years were obtained from the 1934 pruning tests. They indicate that no natural infection takes place after the last week in May or the first week in June. In the case of the Young dewberry, the time of infection is apparently even shorter, for no infection occurred when the primocanes were removed as early as May 12. Some time must elapse between the removal of primocanes and the emergence of the new ones that will be subject to infection; so the date of pruning cannot be taken as the actual limit of the time of infection. It takes about 1 week to 10 days, depending on growing conditions, for new canes to develop sufficiently to be subject to infection.

On the whole, all the species and varieties experimented with behaved alike in regard to the time of infection except the Nanticoke blackberry. On this variety, infection apparently may occur much later than on other varieties, as is shown by the following data. On June 24, 1933, the primocanes of three Nanticoke plants were pruned to the ground, and those of three other plants, situated alternately with the pruned ones in the same row, were left unpruned as checks.

The following spring the control plants showed very severe infection, but the pruned plants also showed severe infection, although decidedly less severe than the controls (approximately 50 percent of the blossoms were diseased as against approximately 90 percent of those of the controls). This was rather surprising when the results obtained with other varieties and species are considered.

In 1934 pruning of primocanes of the Nanticoke was done on June 10 and 30 and August 13, with the following results: On the plants pruned June 10 infection was severe, about the same as on the unpruned controls. On those pruned June 30 infection was very light; of the four plants used, one died, one was entirely free of rosette, and two showed small amounts (two lateral branches on one plant and one on the other). The pruned August 13 showed no infection.

It is apparent from these results that, in the cases where the pruning was done on June 24 and 30, infection must have taken place sometime in July (allowing time for new canes to develop), and this is at least 1 month later than the infection time of any of the other varieties tested.

TIME DURING WHICH INFECTION MAY OCCUR

It is plain from the results of the inoculation, spraying, and pruning experiments that, under Louisiana conditions, the period during which infection takes place is limited. As shown by the spraying and pruning tests (except in the case of the Nanticoke variety) no natural infection occurs beyond the last part of May or the first week in June at the latest. With artificial inoculation, which is a drastic method, infection was obtained as late as the last week in June, and again in the fall, in October and the last part of September. The fall infection is of no practical significance because there is then no inoculum to produce natural infection.

That the limited time of infection is not peculiar to Louisiana conditions is brought out by the results of other workers. Cook (*2*), in Delaware, failed to produce infection from his August inoculations, but he obtained infection very readily by inoculating in June (how late in June is not stated). One of the infection experiments of Reimer and Detjen (*6*) in North Carolina also brings out this fact very plainly. Thirty-two canes of *Rubus nigrobaccus* were inoculated with spore suspension or dry spores at different intervals from February 19 to June 23 and then with pure cultures of the fungus, mycelium and spores, from June 29 to August 5. Sixteen of the canes died after the spore inoculation, but new ones developed in time to be included in the inoculations from June 29 to August 5. This last set of canes remained healthy (except for 1 diseased bud on 1 cane) while the first set of 16 canes were severely infected (72 witches' brooms). These workers offer as explanation of the failure of the canes inoculated from June 29 to August 5 to become infected that "the buds were probably too far advanced for the fungus to secure a foot-hold." This explanation, which would imply that resistance to infection is due to the advanced stage of the growth of the host plant is hardly tenable. Since the new canes developed after June 23, they could not have been in a too far advanced stage of growth when inoculated on June 29. Furthermore, in Louisiana infection has been obtained as late as October 17 (table 2).

Temperature appears to be the chief factor limiting the time of infection. The optimum temperature for the growth of the fungus in culture was found to be about 25° C. The fungus made a fair growth at 27.5° C., but growth stopped at 30° to 31° C. The average mean temperatures for Baton Rouge, La., from May to October for the 5-year period 1931–35 are May, 74.2° F.; June, 79.7°; July, 82.3°; August, 81.8°; September, 78.6°; October, 70.7°. The optimum temperature for the fungus is thus seen to be lower than the mean temperatures prevailing during the period from June to September, the period during which natural infection does not occur. The mean temperature for the month of May, when natural infection does occur, is somewhat below that for the optimum growth of the fungus.

That temperature may not be the only factor operating to limit the time of infection is suggested by the behavior of the Nanticoke blackberry. As shown by the pruning experiments, natural infection apparently occurred on plants of this variety pruned as late as June 30, in which case infection must have taken place in July. It is not clear why this particular variety has a longer period of susceptibility than the other varieties and species tested.

"SYSTEMIC" [5] INFECTION

As stated earlier in this paper, the behavior of one Lucretia dewberry plant in the first spraying experiment (1931) and of a Young dewberry plant in one of the pruning experiments (1933) strongly indicated that, under certain conditions, infection could become systemic in the crown of the dewberry plant. Experimental evidence was secured confirming this.

Dewberries are propagated by tip rootings of the canes. As the cane tip forms roots in the fall, the terminal bud grows out, its basal portion becoming the crown of a young plant and eventually giving rise to secondary canes. When young plants are produced from rosetted canes, the fungus may become established in their crowns and the canes arising from these will be infected.

Twenty-one young plants of the Young variety, produced by tip rooting rosette canes, and 20 similar plants from healthy canes to serve as controls, were planted in the same row on March 10, 1934. When examined in the spring of the following year, two of the controls were found to be dead. All the other controls were healthy. Of the 15 surviving plants from rosette canes, 7 were healthy and 8 diseased. Of the eight diseased plants, every cane on every plant was completely rosetted (every bud) except one. This had one healthy and five rosetted canes.

The plants were pruned to the ground on June 2, 1935, after the time when infection naturally occurs in the spring, in order to see whether the systemic infection of the crown would persist for the second year. In the spring of 1936, the experimental plants presented essentially the same picture as in the previous year. The 18 controls and the 7 plants from rosette canes that were healthy in 1935 were again free of rosette. The eight plants that were rosetted the previous

[5] The term "systemic" is used for lack of a better term. It is realized that the infection is not systemic according to the strict meaning of the word. In typical systemic infections, such as that of the loose smut of wheat, for example, the fungus occurs within the tissue of the host and its growth keeps pace with the growth of the host plant. In dewberry rosette, the fungus does not invade the host tissue but grows externall between the scales of the buds and invades new buds as they develop.

year were again severely rosetted, every cane on every plant being diseased, with the exception of three healthy canes on one plant and two on another.

Although the number of plants used in this experiment was rather small, the results obtained were so definite as to leave hardly any doubt that systemic infection may occur and persist in the crown. Additional information on this phase of the problem was obtained by direct microscopic examination of the buds from systemically infected crowns and also of the buds of rooted tips of rosetted canes.

On March 28, 1934, 13 very young shoots were collected from two Young stools that had shown indications of systemic infection the previous year. These shoots ranged in length from about one-fourth of an inch (just emerging) to about 3 inches. Freehand sections were made and examined. Mycelium was found in great abundance within both the terminal and the lateral buds. Mycelium was not found in the buds of similar material collected from healthy stools.

On the same date (Mar. 28) young shoots were collected from rooted tips of rosetted canes. These tips had formed good root systems and the terminal buds had made considerable growth, extending from 1 to 8 inches above ground. In some, the basal lateral buds had begun to develop to form secondary canes. These shoots were divided into two lots. In one lot was placed the material from tips that were covered with soil to a considerable depth (2 to 5 inches) so that the terminal buds had pushed their way through the soil to emerge, and in the second lot the shoots from tips that formed roots but which were either not covered at all with soil or covered only lightly.

In the second lot, mycelium was found in great abundance both within the terminal and the lateral buds. In the first lot (where the buds remained underground for a considerable time) the results were different. Mycelium was found in seven out of nine terminal and four out of nine lateral buds examined. Furthermore, where mycelium was found in the buds of this material, it was not nearly so abundant as in the buds that were not covered with soil. These findings would indicate that the fungus does not survive for very long underground. It was not known definitely in this case how long the cane tips stayed underground, but judging by the amount of root growth they had made, it must have been 4 to 6 weeks.

The fact that the fungus does not survive very long underground apparently explains the observed phenomenon that some of the plants from tip-rooted rosette canes will have systemically infected crowns while others will have all or a part of their shoots healthy. If the cane tip forms roots as it touches the ground and its terminal bud is not covered with soil, the entire crown of the young plant will be infected. On the other hand, if the tip is covered with soil so that the infected terminal bud stays underground for some time, the fungus may die completely or be killed in some of the basal buds.

In the case of the upright blackberries, systemic infection of the crown does not occur because they are propagated from suckers which develop mostly from the roots. The cane tips of the upright blackberries do not root naturally. If, however, the cane tip is covered with soil and held in this position for some time, it will root. Several rosette canes of the wild blackberry *Rubus abundiflorus* were treated in this manner, and in every case the young plants that developed were healthy.

HISTOLOGICAL STUDIES

In order to determine the relation of the parasite to the host tissues, material was collected at frequent intervals from October to July. This material, which consisted of vegetative and floral buds and flowers at different stages of development, was examined both in the fresh

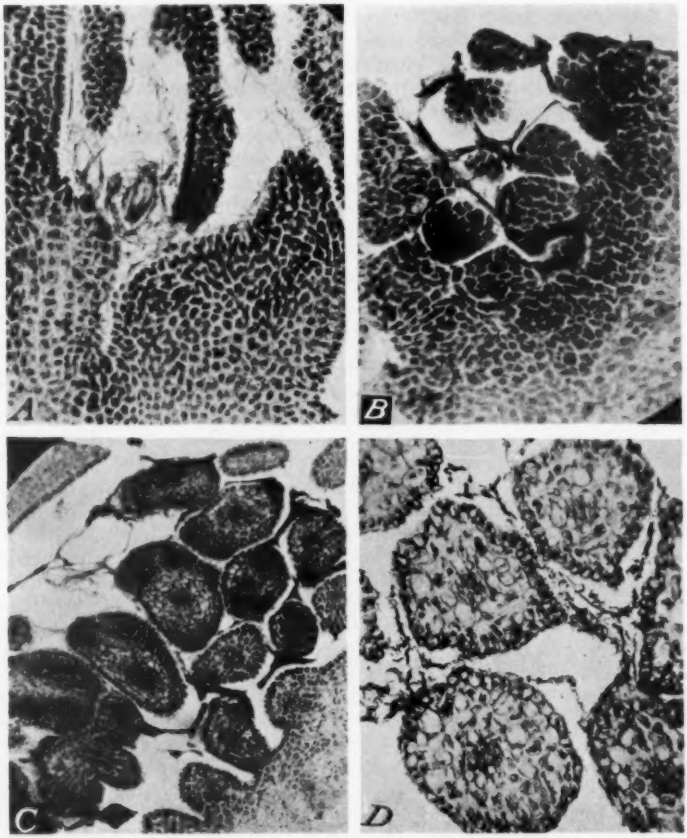

FIGURE 6.—Photomicrograph showing location of mycelium of *Cercosporella rubi* in the host. *A*, Section of vegetative bud of McDonald blackberry showing mycelium between the embryonic elements. × 230. *B*, Section through flower bud of the Young dewberry showing dense network of mycelium between the embryonic blossoms; × 190. *C*, Tangential section through a very young blossom, showing mycelial masses between the embryonic stamens; × 110. *D*, Cross section through a young, unopened blossom of the Lucretia dewberry showing mycelium between the filaments; × 110.

state (freehand sections) and fixed and stained. For the most part, Stoughton's (*8*) thionine-orange G staining method was used, since it gives good differentiation and is fairly rapid, but other stains, especially Heidenhain's iron-alum haematoxylin, were also used.

The fungus occurs in great abundance within both the vegetative and the floral buds (fig. 6). In the spaces between the youngest

embryonic bud elements, the mycelium consists of actively growing, branching, freely anastomosing hyphae, forming a dense network. In the spaces between the older, outer scales of the vegetative buds, the mycelium is less abundant, and, judged by its staining reaction, is in a more or less dormant, semisclerotial stage.

Cook (*2*) states that "small, bud-like, lateral haustoria connect the mycelium with the cells of the host plant." In the Louisiana investigation, however, direct penetration of the living cells either by hyphae or haustoria has not been observed, nor has any intercellular mycelium been found. Thousands of sections of vegetative and floral buds, and flowers at different stages of development, were prepared and examined, both in the fresh state and fixed and stained, but no indication of invasion of the living tissue was ever noted. The only instance in which mycelium was found within the host tissue was in withered, dead anther walls.

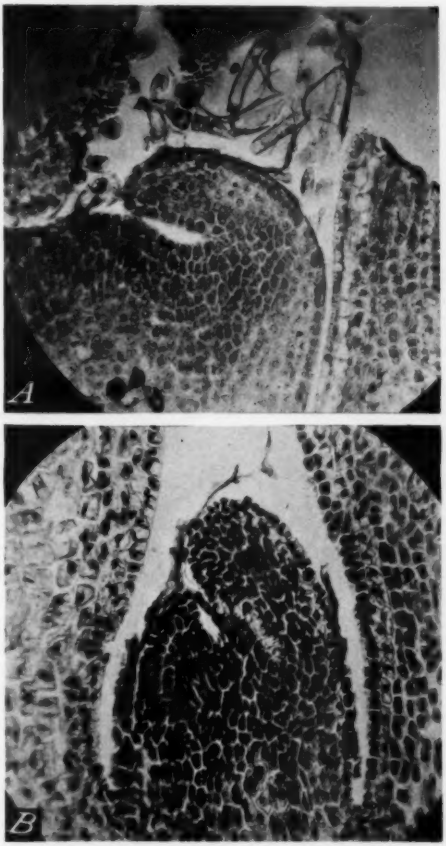

FIGURE 7.—Photomicrograph showing location of mycelium of *Cercosporella rubi* in the host: *A*, Section through a young vegetative bud of *Rubus sons* showing hyphae closely appressed against a leaf primordium and also against the growing point; material collected and killed January 3; × 230. *B*, Longitudinal section through a vegetative bud of the Young dewberry showing mycelial strands in the process of entering a very young lateral bud; × 190.

Since the fungus does not invade the host tissue, the question arises how it obtains its nourishment. It may be that the host tissues exude some nutritive substances which are utilized by the fungus. It seems more probable, however, that the fungus obtains its nourishment by direct absorption through the thin walls of the embryonic cells with which it is in intimate contact. The following observations support this view: (1) Most of the mycelium within the bud is found in the immediate vicinity of the youngest, often undifferentiated tissues. (2) Young hyphae are often found closely appressed against the young, undifferentiated host cells (fig. 7, *A*). (3) The mycelium next to the younger tissue appears to be in an actively growing state, as is shown by its staining reaction. (4) In general very little my-

celium occurs between the outer (older) bud scales, and that which occurs there appears to be in a dormant or inactive stage, the hyphae being cemented together by means of a gelatinous substance in a compact, sclerotiumlike mass.

Large masses of mycelium occur within the ovary cavity and also within the ovule. The occurrence of the fungus within the ovary cavity presents an interesting phenomenon which was rather puzzling when first observed. In the infected flower buds there is a channel through the ovary wall (fig. 9, *B* and *C*) with hyphal strands connecting the mycelium within the cavity with that on the outside. Cook (*2*) states that the fungus "penetrates the ovaries through the stigma, passes down the style, and produces an abundant growth within the ovary." This seems to be an incorrect interpretation of an interesting phenomenon. As a matter of fact, no penetration of the ovary occurs. The fungus is present before the ovaries have begun to develop, even before the inception of the floral bud itself. When the ovary begins to develop, its folds surround the fungus and include it within the ovary cavity. So, instead of "penetration" of the ovary, there is in reality an "enveloping" of the fungus by the ovarian tissues.

For a better understanding of how the mycelium is included within the ovary cavity, the development of the normal ovaries in the blackberry may be briefly described. The ovary primordium appears as a solid protuberance on the receptacle (fig. 8, *A*). Soon this primordium begins to invaginate, the opposite carpellate walls growing around and meeting to form the ovary cavity (fig. 8, *B*). When the carpellate walls meet, there is no immediate fusion of their tissues to seal the ovary cavity; instead, a small opening, the stylar canal (fig. 8, *C*) is left which persists for some time. About this time, the ovule primordia begin to protrude from the inner carpellate walls on opposite sides of the stylar canal (fig. 8, *D*). Two ovules are formed, one developing and the other becoming abortive. The carpellate wall on one side of the stylar canal elongates to form the style. As the ovary continues its development, the cells surrounding the stylar canal fuse (fig. 8, *E*), so that in the mature ovary there is no opening from the cavity to the outside (fig. 8, *F*), only a small notch or scar remaining at the base of the style on the concave side of the pistil to indicate the point of fusion of the carpellate walls.

In the infected (rosette) flower, as the ovary primordium begins to invaginate, the mycelium grows and follows the folds of the developing carpellate walls (fig. 9, *A*) so that when the walls meet the mycelium is enveloped within the ovary cavity, and it also fills the stylar canal. The presence of the fungus in the stylar canal prevents the fusion of the walls, and so in the mature pistils of the infected flower the stylar canal remains as a rather wide opening in the ovary wall (fig. 9, *B*, *C*). This opening is filled with mycelial strands connecting the mycelium within the ovary cavity with that on the outside (fig. 9, *C*). In a similar manner, in the course of the development of the ovule, the integuments envelop the fungus, so that the hyphal strands connecting the mycelium within the ovule with that in the ovary cavity protrude through the micropyle (fig. 9, *D*).

The manner in which the fungus affects the host to cause it to produce the rosette or witches'-broom type of growth cannot be explained with certainty at this time, but it is probably a matter of nutritional deficiency. Since the fungus lives and grows in close contact with the

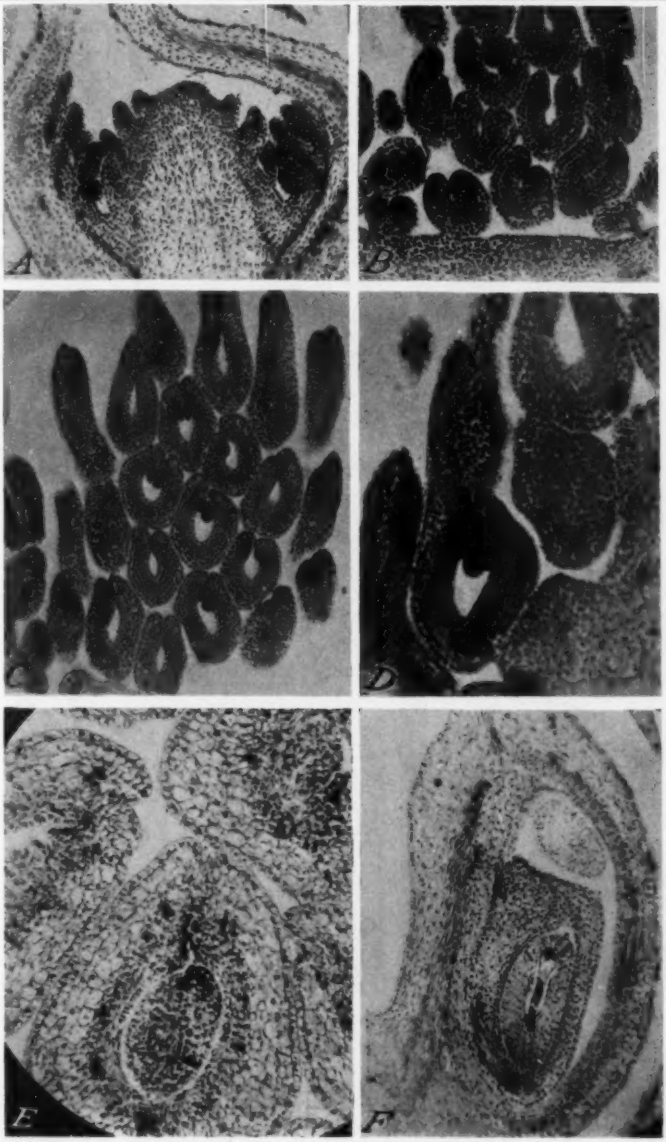

FIGURE 8.—Development of the normal ovary of the blackberry: *A*, Section through a very young blossom showing the pistil primordia as solid protuberances on the receptacle; × 60. *B*, Section through a young blossom at a later stage of development than in *A*, showing the invagination in the pistil primordia to form the ovary cavity; × 103. *C*, Later stage of development than that shown in *B*. The carpellate walls have met and the ovule primordia are beginning to protrude from the inside of the carpellate walls on opposite sides of the stylar canal; × 75. *D*, Later stage than that shown in *C*. One side of the carpel has elongated to form the style. The walls of the stylar canal are closely appressed against each other, but fusion of the tissues had not yet taken place; the stylar canal is still discernible; × 140. *E*, Later stage than that shown in *D*. Section through the scar of the stylar canal, showing the point where the tissues have united, thus sealing the ovary cavity. Only a notch remains at the point of the fusion of the tissues; × 110. *F*, Fully developed ovary. No opening is left in the ovary wall; × 60.

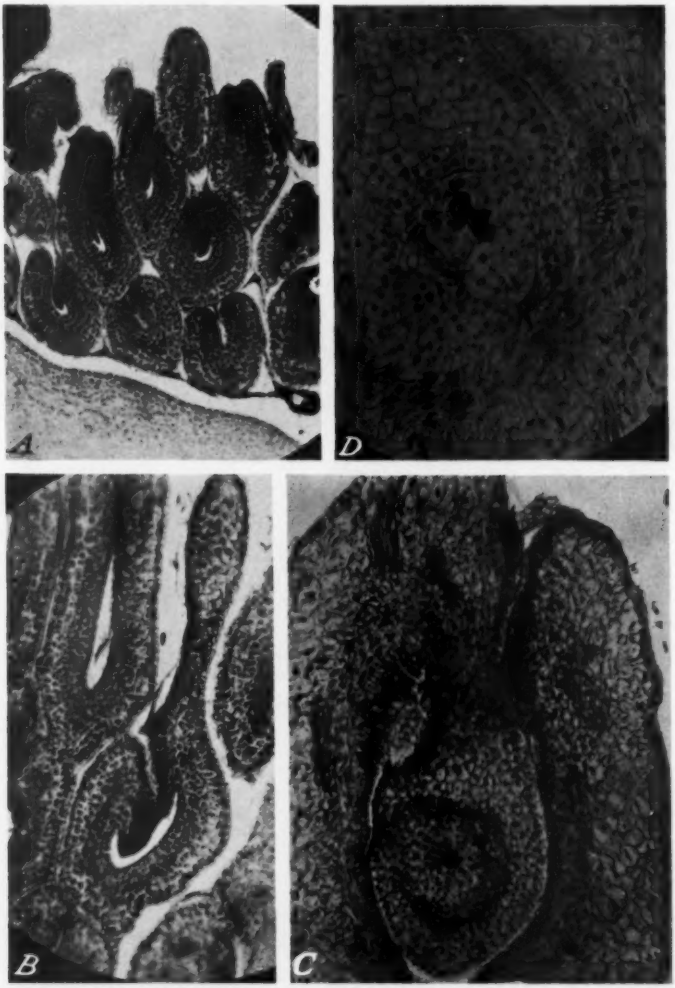

FIGURE 9.—Development of the ovary in the rosette blossom: *A*, Section through a very young rosette blossom of approximately the same stage of development as that shown in figure 8, *B*. Masses of mycelium may be seen between and also within the invaginations of the embryonic pistils; × 115. *B*, Later stage of development than in *A*. One side of the carpellate wall has elongated to form the style. The stylar canal with mycelial strands in it is plainly visible; × 130. *C*, Final stage; fully developed ovary. The mycelium within the stylar canal has prevented the walls from uniting, and thus a prominent opening is left; × 108. *D*, Section through an ovule showing mycelial strands protruding through the micropyle; × 220.

embryonic tissues of the bud, it must absorb and utilize nutritive substances that are necessary for the normal development of the bud, and the growth of the latter is checked. When the growth of the first or terminal bud is interfered with, secondary buds begin to develop at the base, but these are also invaded by the fungus (fig. 7, *B*) and their

development is checked. The result is the production of a large number of secondary buds and the formation of the rosette type of growth.

The possibility that the fungus may secrete some toxic substance or substances within the bud which has a deleterious effect on the host cells, thus interfering with normal growth, is not overlooked, although this cannot be easily proved or disproved. The following experiment was performed in an effort to obtain some information on this point.

The fungus was grown for 10 days on bean-pod decoction in 250-cc flasks. The mycelium was macerated in a mortar and mixed with the decoction on which it had grown, and the mixture was filtered through a porcelain bacterial filter. Small, funnel-shaped, paraffined paper cups were cemented, by means of melted paraffin, around fruit buds of Young dewberry plants, and enough of the filtrate was placed in each cup to submerge the bud. Each bud was punctured once with a needle, and its scales were teased open to some extent to allow the liquid to penetrate. Twenty-seven buds were treated in this manner, and 19 others were used as checks. In the case of the checks, sterilized bean-pod decoction was placed in the cups. The buds were left submerged for 26 hours. The experiment was started on March 9, 1936. At this time the buds selected for the experiment were swollen preliminary to opening. The results of the experiment were negative; no rosette symptoms developed. Both the checks and the buds treated with the fungus filtrate grew normally and produced fruit.

It is realized, of course, that the fact that this test gave negative results does not prove that the fungus filtrate did not contain some toxic principle, for the time element must be considered. The filtrate remained in contact with the bud only 26 hours, whereas in the case of infected buds the fungus is in contact with the host tissues for a very long time.

SUMMARY

This paper presents the results of a study of the rosette disease of blackberries and dewberries and its causal agent.

The rosette disease was found to be identical with the "double blossom" disease described earlier by Cook and others.

The geographic region in which the disease occurs, so far as is known, includes all the southern and southeastern sections of the United States, from New Jersey to southeastern Texas, and from southern Illinois to the Gulf of Mexico.

The causal fungus, which was first described and named *Fusisporium* (?) *rubi* by Winter and later placed in the genus *Ramularia* by Wollenweber, more properly belongs in the genus *Cercosporella*, and so it has been renamed *Cercosporella rubi* (Winter) comb. n. A revised technical description is given.

The spores of the fungus have been found to vary in size, in shape, and in number of septa. The fungus grows readily on a variety of media and sporulates fairly well in culture. The minimum, optimum, and maximum temperatures for growth in culture were found to be about 6°, 25°, and 30° C., respectively.

The mycelium is found within the vegetative and flower buds of the host, but sporulates only on the open blossoms in the spring. Ordinarily infected primocanes show no external symptoms, but on

resuming growth the following spring, the infected buds form the rosette or witches'-broom type of growth.

The pathogenicity of *Cercosporella rubi* has been established by repeated inoculation experiments over a 4-year period, 1931–34. Infection was obtained from inoculations on wild and cultivated species and varieties of blackberries and dewberries.

It has been determined by inoculation, spraying, and pruning experiments that the period during which infection takes place is limited. Under Louisiana conditions, natural infection does not usually occur after the last week in May or the first week in June. It is believed that temperature is the chief factor limiting the time of infection, as the prevailing summer temperatures for Louisiana are higher than the optimum temperature for the fungus. On the Nanticoke blackberry, however, infection has been found to occur as late as July. This suggests that other factors besides temperature may have a determining influence on the time of infection.

Histological studies have shown that the mycelium occurs in great abundance within the vegetative and flower buds. No invasion of the tissue has been observed, but the fungus has been found in close association with the embryonic bud elements, and it is thought that it obtains its nourishment by direct absorption through the thin walls of the young cells. It is believed that the parasite causes the disease by starving the infected buds. As the fungus absorbs the substances that are necessary for the normal development of the bud, the growth of the infected bud is hindered and basal buds begin to develop. These in turn are invaded by the fungus and their normal development is inhibited. This results in the production of a rosette type of growth.

In the infected pistils, the fungus prevents the fusion of the carpellate walls so that a prominent channel, the stylar canal, is left in the carpel wall at the base of the style. This canal is filled with hyphal strands which connect the mycelium within the ovary cavity with that on the outside. Mycelium occurs also within the ovule.

Experimental evidence was obtained showing that infection may become systemic under certain conditions in the crowns of dewberry plants arising from tip rootings of rosetted canes. This does not occur in the case of the upright blackberries.

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A STUDY OF SCAB RESISTANCE IN THE POTATO[1]

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INTRODUCTION

Scab, caused by *Actinomyces scabies* (Thax.) Güssow, is one of the most common and widespread diseases of potato (*Solanum tuberosum* L.). Various methods of control, involving seed treatments, soil treatments, and crop rotation, have been recommended but no method or combination of methods has proved very satisfactory. Scab is still one of the most important limiting factors in the production of high-quality potatoes.

Of the potato varieties grown commercially in the United States only the Russet Burbank and the Russet Rural are appreciably resistant to scab. These varieties have been used extensively where it is necessary to grow potatoes in infested soil, but their adaptability is very limited, and they are entirely unsuited for many types of potato culture. Both varieties mature late and are poorly adapted to peat soils. For these reasons their usefulness in scab control is limited. The unsatisfactory nature of all the available control measures has led to a strong demand for new scab-resistant varieties adapted to different soil types and conditions of growth.

In 1931 Krantz and Leach (*8*)[3] emphasized the need for a national potato-improvement program and pointed out that relatively little work had been done in breeding for disease-resistant varieties in the United States. In 1934 Stevenson and Clark (*23*) reported progress on a cooperative project in which particular attention was directed toward breeding varieties resistant to late blight and to some of the more destructive virus diseases. In the same year Reddick (*16*) reported encouraging progress in breeding for resistance to late blight. In Europe, breeding for disease resistance in potatoes has dealt primarily with resistance to black wart (*9*, *12*, *22*) and late blight (*15*), although in recent years some work on scab resistance has been reported by Berkner (*2*) in Germany and by Lunden (*11*) in Norway. Schlumberger (*20*, *21*) has tested numerous new varieties produced by commercial breeders and has reported several of them to be scab-resistant.

The present study was undertaken as part of a cooperative project on the production of disease-resistant potato varieties, and deals specifically with common scab. The objects of the investigation were (1) To determine the degree and variation of scab resistance in potato seedlings of both inbred and hybrid groups which were being used in an extensive potato-breeding program, with a view to using resistant

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[2] The writer wishes to express his appreciation to Dr. J. G. Leach, under whose direction the work was done, for helpful criticism, and to Dr. F. A. Krantz for valuable advice and for furnishing the potato seedlings used in the experiments.

[3] Reference is made by number (italic) to Literature Cited, p. 316.

ones as parental material in developing scab-resistant varieties of commercial value; (2) to study the breeding behavior of certain inbred scab-resistant seedlings and (3) insofar as possible to determine the nature of scab resistance.

MATERIALS AND METHODS

The potato seedlings used in this study were produced in the course of a general potato-breeding project that had been in progress at the Minnesota Agricultural Experiment Station since 1920. The tests were made on plots of scab-infested soil at the following places: In 1931, at Coon Creek and University Farm, St. Paul, Minn.; in 1932, at Coon Creek and University Farm and at Brooklyn Center, Minn., about 5 miles north of Minneapolis; in 1933 and 1934, at Coon Creek.

The soil of the University Farm plot is a silt loam and was not satisfactory because very little typical scab developed. The soil at Coon Creek is a high-lime peat and is heavily infested with scab. The soil at Brooklyn Center is a light sand. In 1932 a very heavy infection of scab was obtained at Brooklyn Center, but in 1933 it was necessary to discontinue the test there. Standard varieties, known to be susceptible to scab, were planted along with the seedlings as a basis for comparison.

The plan of each plot was practically the same at all locations. Seedlings were duplicated in each plot whenever possible. The relative amount of scab on each seedling was given a rating of heavy (H), medium (M), or light (L), depending upon the amount of scab on all of the tubers of the seedling. The susceptible standard varieties, Early Ohio, Bliss Triumph, and Irish Cobbler, were used for comparison. For all practical purposes this method proved satisfactory for selecting the resistant types. All selections rated H and most of those rated M were considered as susceptible and were usually discarded from the test. Those rated L and some of those rated M were considered as possibly resistant and were saved for further testing. New selections were added each year to replace the discarded ones. Thus all selections have not been tested for the same number of years. Some selections rated L in the first year's test proved susceptible in later tests. This occurred mostly when the tubers of the first year's test were very small and developed very late in the season. Such tubers apparently escape infection more readily than larger tubers formed normally. For convenience in this paper, selections that were rated L are spoken of as resistant, although none of them are immune and it is realized that subsequent tests may prove some of them to be susceptible. Only those selections rated L over a series of years are considered worthy of use as breeding material, and these will be given more extensive tests for resistance.

The material used in the tests consisted of inbred selections, hybrid selections, seedling families of inbred selections, and F_1 seedling families. By making the tests on seedlings that have been selfed for a number of generations and are more nearly homozygous, the value of scab-resistant selections as breeding stock is greatly increased. Moreover, by selecting from inbred populations those seedlings which set seed, fertile breeding stock is more likely to be obtained (*6*, *7*).

At the beginning of the experiment it was recognized that differences in time of tuber formation may effect the amount of scab that will subsequently develop. Sanford (*18*, *19*) has shown that scab infection

is limited to a period of approximately 10 days after tubers begin to set and that high soil moisture at this time inhibits infection. If there were wide variation in the time of tuberization, some of the seedlings might be forming tubers during a rainy period and so escape infection. To test this possibility, both early and late susceptible varieties were planted at four successive 12-day intervals, beginning May 26, 1931. The entire plot was harvested in late September, and no apparent difference in the amount of scab was observed. From these results it was concluded that there was not sufficient variation in the normal

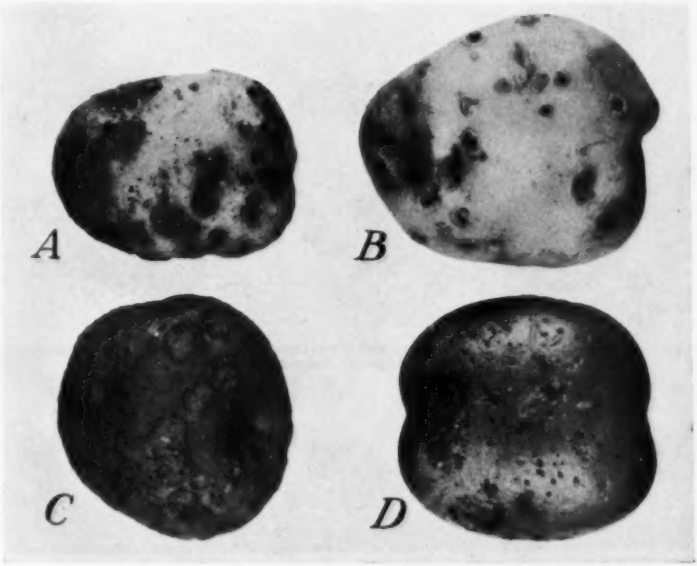

FIGURE 1.—Representative average reaction to scab of the standard potato varieties used as checks: *A*, Green Mountain; *B*, Irish Cobbler; *C*, Triumph; *D*, Russet Rural.

soil moisture of the plot to inhibit scab development on susceptible varieties during the season of 1931, regardless of the date of tuberization. This experiment was made on the Coon Creek plot where the peat soil is several feet deep and is well drained to maintain uniform moisture conditions. The soil is heavily infested with the scab pathogen, and uniformly heavy infection always develops on susceptible varieties.

RESULTS OF TESTS FOR SCAB RESISTANCE

A large amount of inbred seedling stock was available for testing when this investigation was started in 1931. The more promising of the resistant seedlings were selected after each test, and new seedlings were added to take the place of those discarded. Approximately 300 inbred seedlings were tested during the 4-year period 1931 to 1934, inclusive. Twenty-four of these were classed as resistant; 17 are listed in table 1 along with the standard varieties used as checks.

The representative reaction of certain inbred seedlings and the standard varieties are shown in figures 1, 2, and 3. Unfortunately,

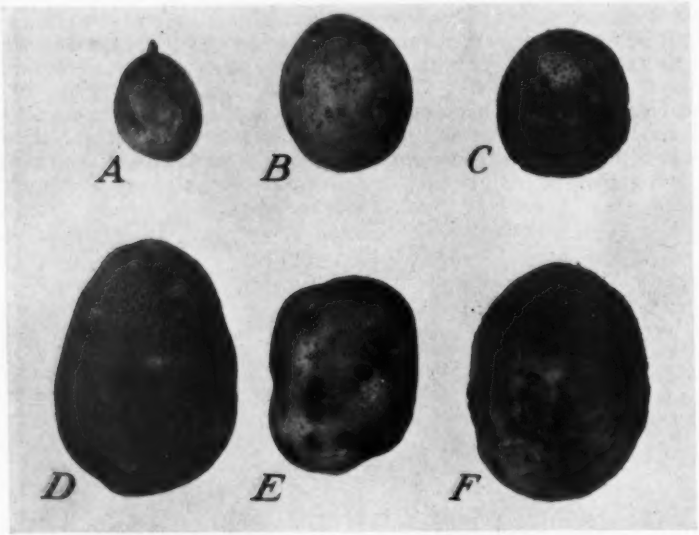

FIGURE 2.—Representative average reaction to scab of five inbred seedlings (*A–C*, *E*, and *F*) as compared with that of the Russet Burbank variety (*D*).

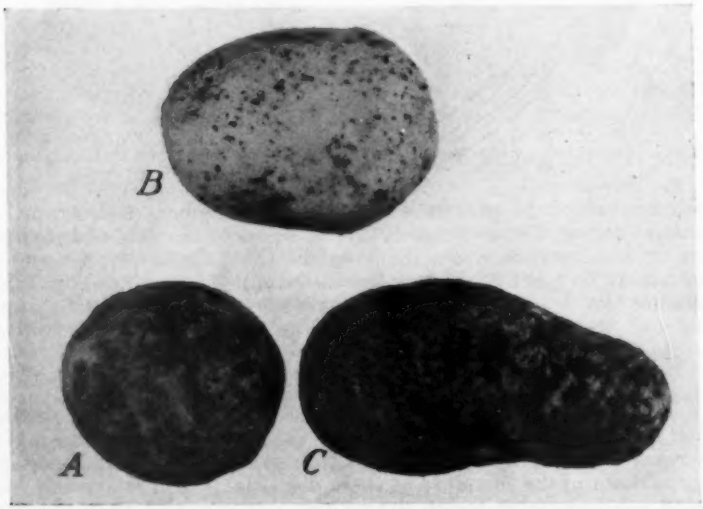

FIGURE 3.—Three tubers, each representing a different F_1 hybrid from the same cross, show wide variation in scab resistance: *A*, 28.26-2-30; *B*, 28.26-6-30; *C*, 28.26-5-30. For origin of hybrids see table 1.

the reaction to scab of some of the varieties from which the seedlings originated is not known. However, one of the resistant seedlings originated from the Irish Cobbler and three others from the Early Ohio. Both of these varieties are quite susceptible. The results of these tests indicate that resistant seedlings may be obtained by inbreeding, even from susceptible varieties.

Tests also were made of a considerable number of F_1 hybrids from crosses originally made for the purpose of combining characters without regard to scab resistance. Most of these hybrids were being tested for commercial possibilities. During the years 1932 to 1934 about 140 were tested. Since many of the F_1 seedlings were susceptible they were discarded from the scab plot, and at the present time tests are being continued on only a few of the more promising ones. In table 1 are listed a number of these F_1 hybrids showing the variation in scab resistance. It will be noted that there is often a wide range in scab resistance among seedlings of the same parentage. A striking example of this is shown in figure 3.

TABLE 1.—*Reaction to scab of certain inbred and F_1 seedlings and standard varieties*

Seedling no. or variety	Years tested	Origin of line	Reaction to scab.[2]
	Number	**INBRED SEEDLINGS**[1]	
4-11-1-2-6-1	2	F_1 seedling of Keeper × Silverskin	L
4-14-2-1	1	do	L
4-65	2	do	L
5-10-1	2	F_1 seedling of Peerless × Lookout Mountain	L
5-14	4	do	L
5-14-8-1	2	do	L
11-4-7-17	2	F_1 seedling of 41-17 × 4-9-1[3]	L–M
12-1-2	3	Snowflake	L–M
12-2-1	2	do	L
22-1	2	F_1 seedling of McCormick × 4-27[3]	L
29-9-2	2	F_1 seedling of 41-1 × 4-25[3]	L–M
30-11-3	2	F_1 seedling of 11-3 × 41-1[3]	L
39-17	2	Irish Cobbler	L
41-1-1-2	2	Early Ohio	L
41-1-1-7-5	2	do	L
41-15	2	do	L
63-58	3	Elk River	L
		F_1 SEEDLINGS[4]	
2.26-4-30	2	Triumph × 41-1[3]	H
2.26-6-30	2	do	H
14.29-1-31	2	11-2-1-21-1 × 11-1[3]	H
15.26-4-30	2	Rural New Yorker × 4-16[3]	L
15.26-6-30	2	do	L–M
18.29-2-31	2	40-3-1 × 21-2-2[3]	M
28.26-2-30	2	10-6 × 11-5[3]	H
28.26-5-30	2	do	M–H
28.26-6-30	1	do	L
39.26-13-30	2	41-1 × 4-25[3]	H
39.26-4-30	2	do	L–M
39.26-5-30	2	do	L
		STANDARD VARIETIES	
Triumph	4		H
Green Mountain	4		H
Russet Rural	4		L–M
Russet Burbank	4		L
Irish Cobbler	4		M–H
Early Ohio	4		H

[1] The first figure of seedling numbers represents the cross or variety which is given under "Origin of line"; the subsequent figures represent selections from successive inbred generations.

[2] L=light infection; M=medium infection; H=heavy infection.

[3] Line 41 is Early Ohio; line 11 is an F_1 seedling of 41-17 × 4-9-1; line 4 is a seedling of Keeper × Silverskin; line 40 is U. S. D. A. 38946; line 21 is an F_1 seedling of 49-1 × 4-9-1; line 49 is an F_1 seedling of Irish Cobbler × Lookout Mountain; line 10 is a seedling of Beauty of Hebron.

[4] The first figure represents the cross which is given under "Origin of line"; the other figures indicate the year the cross was made, the selection, and the year the selection was made, respectively.

To determine to what extent resistance would be inherited, the progeny of certain resistant and certain susceptible seedlings were tested for scab resistance. No statistical study could be made of the results obtained, but it was observed that the progeny of certain susceptible seedlings included both resistant and susceptible seedlings, although the majority were susceptible. Such a population is shown in figure 4. Other susceptible seedlings produced only susceptible progeny. This type is illustrated in figure 5. The progeny of 1 resistant seedling contained 29 seedlings, 26 of which were resistant.

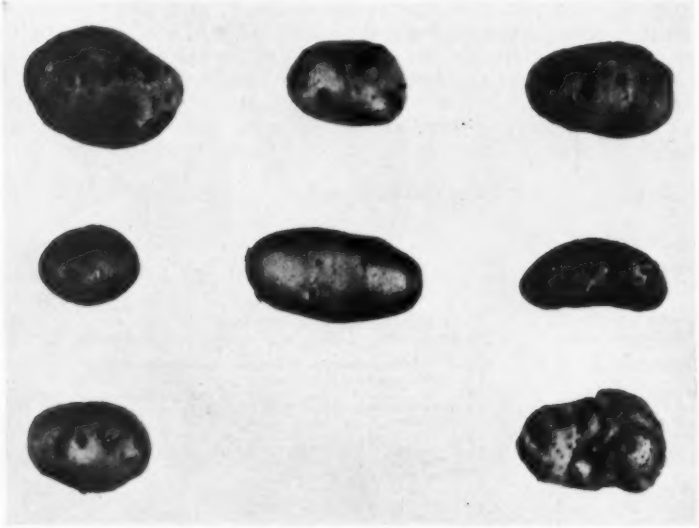

FIGURE 4.—Representative reactions of inbred progeny from a susceptible parent (41-5-5-13), each tuber representing a different plant; note variations in susceptibility.

The remaining three were classed as medium susceptible. Two tubers of this seedling and 1 tuber of each of 12 plants of its inbred progeny are shown in figure 6.

THE NATURE OF SCAB RESISTANCE

Because of the scab resistance of the Russet Burbank and the Russet Rural varieties and the absence of scab resistance in the smooth-skin commercial varieties, it has been suggested that scab resistance may be caused by, or be closely associated with, the russet type of skin (*3*). However, some of the most resistant seedlings found in these tests have smooth skins, a fact which shows that scab resistance is not necessarily associated with a russet skin.

In 1913 Lutman (*13*) stated that scab lesions may originate at any place on the potato but that they frequently occur at the lenticel, and in 1919 he (*14*) declared that "thickness of the skin determines the resistance of the tuber to scab; color seems to play no role in this

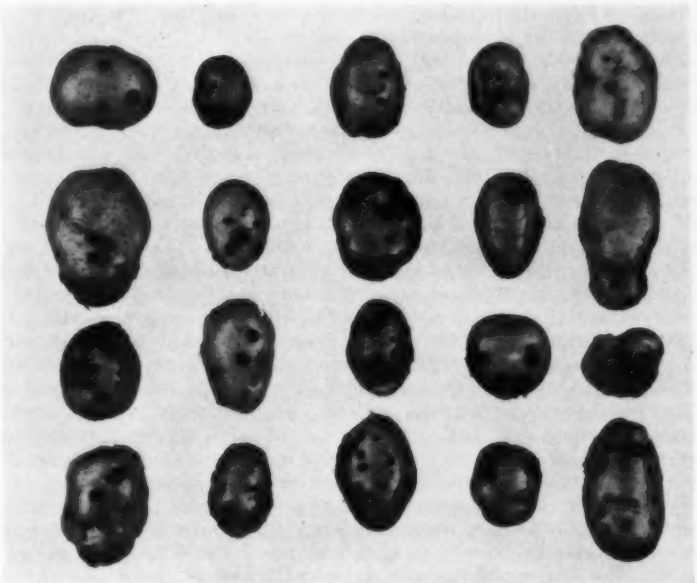

FIGURE 5.—Representative reactions of inbred progeny from a susceptible parent (12-2-4-1), each tuber representing a different plant; note entire population was susceptible.

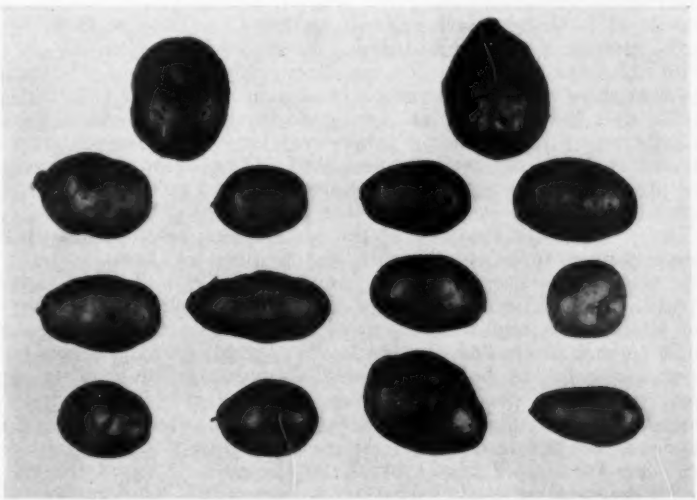

FIGURE 6.—Representative reactions of inbred progeny from a resistant parent (5-10-1), two tubers of which are shown at the top; nearly all the individuals of this population were as resistant as the parent.

resistance." Sanford (*19*), Fellows (*4*), and Longrée (*10*) subsequently presented evidence that the lenticels are the principal avenues of entrance of the scab pathogen.

From a histological study of the various types of scab, Jones (*5*) concluded that the degree of suberization of the lenticels determined the degree of susceptibility to at least one type of scab and that the development of a lesion is more or less restricted by the formation of wound cork beneath the infected areas. Longrée (*10*), in a recent and very critical study, has again emphasized the importance of lenticels in determining penetration. She concluded that the shape and size of the surface of the lenticels are unimportant in determining scab resistance. She pointed out, however, that in the resistant types the complementary cells of the lenticels were round and compact, while in the susceptible types they were longer or more irregular and were loosely associated. She also observed that the lenticel cambium of resistant varieties in most cases became suberized relatively early, while that of the susceptible varieties was suberized relatively late. In addition, she noted that in the later stages of tuber development susceptible varieties formed wound cork profusely, while resistant varieties formed very little. Thus, infection might occur in resistant varieties of this type, and yet the scab lesions would remain small or disappear entirely because less wound cork was formed.

Wingerberg (*24*) maintains that the suberization of the lenticels and their ability to form wound cork is of no importance in determining resistance, and he concludes that resistance rests on a physiological basis. The nature of the physiological reaction, however, was not determined.

As comparative material for the present study on the nature of scab resistance, a susceptible and a resistant seedling were selected. The resistant seedling was 5–10–1 (fig. 6), and the susceptible seedling was 1sc of 12–2–4–1 which is shown in figure 5. Both of these seedlings produce smooth-skin tubers. Since lenticels seem to be the point of infection for scab, a comparative study of the lenticel development of these types was made. Inasmuch as Sanford (*18*, *19*) had shown that the first 10 days of tuber formation is the critical period of infection, young growing tubers not larger than one-quarter to one-half inch in diameter were compared. The tubers were harvested and preserved in 70-percent alcohol, sectioned on a sliding microtome, stained with Sudan IV, and mounted in glycerin.

The writer's observations of the histological development of the young potato agree closely with the findings of Artschwager (*1*). The tuber originates as a stolon from an axillary bud on the underground stem. The young tuber begins to develop on the tip of the stolon as a slight radial swelling and gradually develops the characteristic shape of the variety. Apical growth occurs, the tuber increasing in both length and diameter. The growing point gives rise to a single-layer epidermis, and certain cells become specialized to form stomata. Because of the subsequent development of the periderm, these stomata are only temporary, and new ones are formed very sparsely on the growing tip of the tuber. With the formation of the periderm underneath the epidermis certain cells below the stomata begin to divide actively, producing a mass of more or less spherical cells from which the protoplast soon disappears,

the cell walls becoming suberized in the process. As the number of these cells increases they rupture the epidermis, and the lenticel is thus formed. When the tuber is about one-quarter inch in diameter a considerable part of the epidermis has already been sloughed off and is replaced by the periderm.

The stomata of many specimens of the resistant and susceptible types were examined, and it was concluded that the stomata of the two types are essentially alike. In all cases they were found to be short-lived, functioning only during the very early stages of tuber formation. It is not likely that the character of the stomata influences the resistance of a variety.

Macroscopic examination is sufficient to show that, in general, the lenticels of the susceptible seedlings used in this study were larger than those of the resistant seedlings. (Compare the lenticels of the seedling shown in fig. 5 with those of the seedling in fig. 6.)

A tuber of seedling 12–2–4–1 (shown at the extreme upper left corner of fig. 5) and one of the tubers of seedling 5–10–1 (shown at the top of fig. 6) were grown and young tubers of the new crop were used for a more detailed study of the lenticels in relation to scab resistance. Figure 7 shows photomicrographs of sections of representative lenticels from young tubers cut when the tubers were about one-quarter inch in diameter. Here, again, the contrast in the size of the lenticels is very striking. But this is not all. The lenticel cells of the resistant seedling 5–10–1 (fig. 7, *A*) are small and closely associated into a compact unit, with very small intercellular spaces. The structure begins abruptly at the starch parenchyma and extends outwardly as a narrow column of somewhat rectangular oblong cells. These cells are empty and their walls are brown and thickened. They extend through the periderm to the surface, where a few of the more superficial ones are sloughed off. These are the so-called complementary cells and their compact association is maintained throughout.

When the structure of the lenticel of the susceptible seedling 12–2–4–1–1sc (fig. 7, *B*) is examined the difference is obvious. The lenticel is not only much larger but it is very different in structure. Its cells are more nearly spherical and less closely associated. They begin at the starch parenchyma and extend upward and slightly outward so that the lenticel at the surface is considerably wider than at the base. At the surface of the lenticel the complementary cells are obviously more loosely arranged than in the resistant type. Such a lenticel would appear to constitute a more suitable avenue of entrance since its structure furnishes less protection for the meristematic tissues.

There is also evident a difference in the degree of suberization of the periderm as well as of the more external cells of the lenticel. In the resistant seedling 5–10–1 (fig. 7, *A*) a well-suberized layer about three cells thick extends throughout the periderm while the tuber is still quite young. In the susceptible seedling very little suberization has taken place. The formation of this barrier would be of no significance if the cells of the lenticel were not suberized at the same relative level as those of the periderm and at approximately the same time. When this occurs, as it does in the resistant seedling, a well-defined and more or less continuous suberized barrier is formed over the entire surface of the tuber.

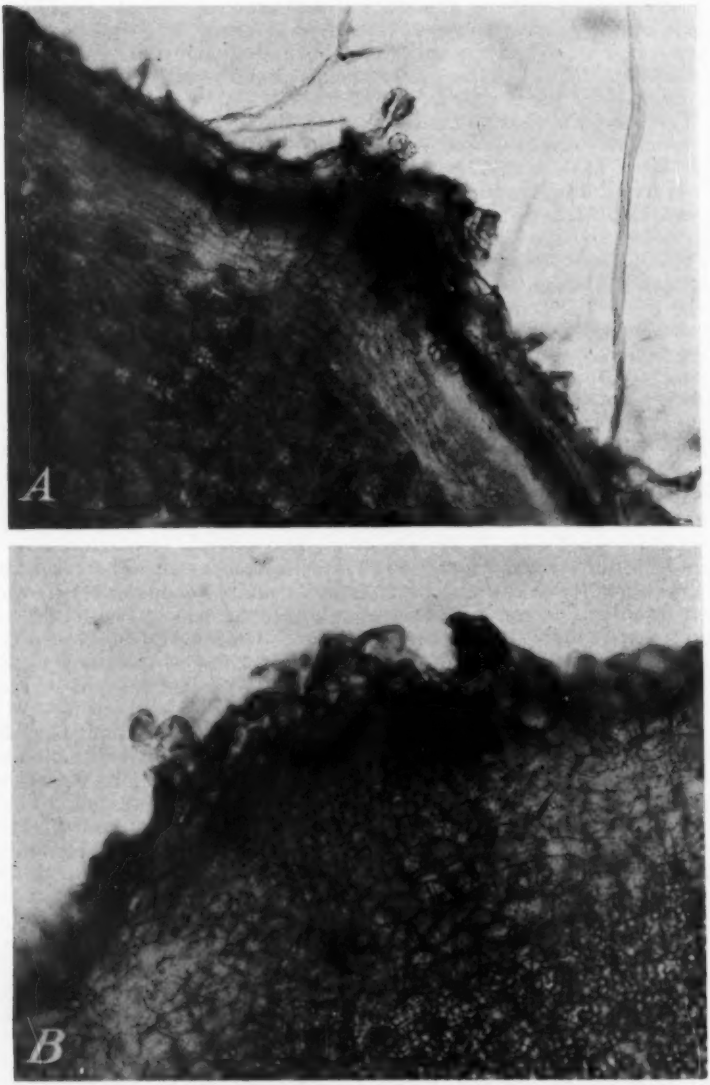

FIGURE 7.—*A*, Section of lenticel of a resistant seedling (5-10-1) taken from a tuber about one-quarter inch in diameter; note close association of the somewhat oblong cells, the dark layer extending through the lenticel being suberized tissue stained with Sudan IV. ×98. *B*, Section of lenticel of a susceptible seedling (12-2-4-1sc) made from a tuber about one-quarter inch in diameter; note the round lenticel cells loosely associated and compare size of lenticel with that shown in *A*, this section having also been stained with Sudan IV. ×98.

DISCUSSION

In the studies reported above resistance to scab has been demonstrated in a number of seedlings produced by selfing or crossing commercial varieties and certain seedlings. Resistant seedlings were found in the progeny of susceptible parents, indicating that, although most commercial varieties are susceptible, they may contain factors for resistance. By selfing, plants having these factors may be isolated in a relatively homozygous condition. This fact is illustrated by the inheritance of resistance in a very high percentage of the progeny of the resistant seedling 5–10–1.

Another significant result is the demonstration that resistance is not always associated with the thick or russet-skin tubers. Many of the resistant seedlings had smooth skins. There was also no association of tuber color or shape with scab resistance. The results of this study, although incomplete, strongly indicate that it should be possible, by crossing the proper inbred seedlings and selecting from the progeny, to combine scab resistance with desirable commercial qualities. It is realized, however, that the problem is a complex one. Even after desirable parental material has been selected, the difficulties of obtaining desirable commercial varieties are numerous. Nevertheless, the outlook is promising. It is felt that fair foundation stock of scab-resistant material within the species *Solanum tuberosum* has been obtained and that the value and practicability of inbreeding and testing within the cultivated potato as a means of isolating scab-resistant types has been proved.

Insofar as the study of the nature of scab resistance has been carried, the evidence points towards the structure of the lenticel as a determining character although other factors may be involved. In the absence of a sustained wound the lenticel seems to be the principal point of infection. The lenticels of the resistant seedling studied were much smaller than those of the susceptible seedling. Their cells were also smaller, more compact, and oblong. It is significant that this character was transmitted to a majority of the inbred progeny of the resistant seedling, 5–10–1, indicating its probable value as scab-resistant breeding stock.

In studying the nature of scab resistance the importance of such soil factors as physical composition, moisture, temperature, fertility, hydrogen-ion concentration, and microflora must be carefully considered. Although scab in itself is dependent upon the presence of the pathogen in a growing tuber, the disease is complexly interrelated with its environment. Scab resistance apparently is only relative, and it is doubtful whether complete immunity exists. The Russet Burbank variety, for example is usually rated as highly resistant, but under certain conditions it may be susceptible. The russet seedling U. S. D. A. 44537 is reported as highly resistant in some localities (*23*), but in the test plot used in these studies it scabbed heavily. Similar variations have been observed in a few other seedlings which have been tested in different localities. It is possible that this may be due to different physiologic forms of *Actinomyces scabies*. This possibility should be investigated.

SUMMARY

Lack of suitable control measures for potato scab has led to a demand for scab-resistant varieties.

Inbred and hybrid seedlings were tested on scab-infested soil, and a number of resistant seedlings were found in each group.

The progeny of certain inbred resistant seedlings were nearly all resistant, while the progeny of certain inbred susceptible seedlings were all susceptible. Certain other inbred susceptible seedlings produced both susceptible and resistant progeny.

Scab resistance is apparently relative and complete immunity has not been found.

There was no consistent association of scab resistance with a russet skin.

Although it is possible that infection may occur through stomata, the lenticel appears to be the chief point of infection. No differences were found between stomata of resistant and susceptible seedlings.

The lenticel structure of a susceptible and a resistant seedling was studied. The lenticels of the susceptible seedling were larger than those of the resistant seedling and the cells were rounder and more loosely arranged, thus furnishing a better infection court.

In the resistant seedling the periderm was suberized earlier than in the susceptible and the suberization extended farther into the lenticels apparently affording greater protection against infection of the meristematic tissues by the scab pathogen.

The occurrence of scab-resistant seedlings, and their breeding behavior as indicated in a limited number of progeny tests, lends encouragement to the belief that scab-resistant varieties of desirable commercial character may be produced.

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